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THE UNIVERSITY OF ALBERTA

THE ASSAY OF ANTIBIOTICS IN PHARMACEUTICAL PREPARATIONS BY POLAROGRAPHY

by

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A THESIS

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The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies for acceptance, a thesis entitled "The assay of antibiotics in pharmaceutical preparations by Polarography", submitted by Muhammad Abdur Rashid Purkaystha in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

ABSTRACT

Rapid and simple polarographic methods are presented for qualitative and quantitative evaluation of various groups of antibiotics. The groups studied were tetracyclines, streptomycins, chloramphenicol, neomycin sulphate, erythromycins, penicillins, oleandomycins, bacitracin and polymyxin. It was found possible to differentiate between groups of antibiotics and also to differentiate between individual members of the same group by observation of the half-wave potential in the individual circumstances.

The validity of the polarographic determination as a measure of the potency of most antibiotics has been established by comparative microbiological assay and comparison of degraded antibiotics. It was not possible because of the unavailability of a suitable organism to compare bacitracin. Chloramphenicol could not be degraded by stages by the method used for degradation and comparison was not possible. Since the polarographic technique to measure penicillins involved complete hydrolysis to inactive materials, microbiological comparison could not be carried out for this group of antibiotics.

The polarographic methods under appropriate conditions have been successfully applied to the quantitative evaluation of antibiotics present in a number of commercial pharmaceutical preparations. Certain representative pharmaceutical preparations were examined and both qualitative and quantitative measurements were made in the following antibiotics: tetracycline hydrochloride, chlorotetracycline hydrochloride, demethyl chlorotetracycline hydrochloride, oxytetracycline hydrochloride, calcium diterramycin, chloramphenicol, erythromycin ethyl succinate and triacetyl

oleandomycin. The pharmaceutical preparations used were in a variety of dosage forms such as tablets, capsules, ointments, syrups, powder and injectable solutions.

In pharmaceutical preparations, a careful evaluation of the effect of known as well as possible added materials,solvents, etc. was made and the results are reported. It was found that lactose, sucrose, glucosamine, N-acetyl glucosamine, magnesium stearate,stearic acid, boric acid, calcium sulphate, starch, methyl paraben, propyl paraben, citric acid, caffeine and sodium citrate did not affect the quantitative measurement of the antibiotics under the conditions used. In those cases where there was slight rise or wave in the regions of the waves of the antibiotics, either the effect was insignificant because of their very low proportion in the pharmaceutical preparations or could be eliminated satisfactorily. In no case was it impossible to eliminate the interference for satisfactory measurement of the antibiotics.

The significance of the polarographic method to the quantitative analysis of the pharmaceutical preparations is discussed.

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I

INTRODUCTION

The therapeutic importance of the antibiotics in the treatment of infectious diseases of man is widely known. Increasingly they are also being used for treating animal diseases; and their inclusion in animal feeds has become common practice. The use of antibiotic drugs more recently for plant diseases has resulted in the position of antibiotics becoming universal in the field of chemotherapy. New antibiotics continue to be discovered which are effective in the treatment of a variety of conditions and exhibit greater degrees of specificity.

Numerous methods for the assay of antibiotics have been described in the literature (1,14,36,54,67 and 91) and there have been many modifications of these various methods (15,24,44,45,59 and 74). It is apparent that there is no single method which has been found to be applicable to the estimation of antibiotics as a class of drugs.

The various microbiological methods as well as most of the chemical methods which have been used are very lengthy and tedious. Some are non-specific, which becomes particularly important when mixtures or pharmaceutical preparations are to be analysed. Microbiological methods, it is recognized, suffer from a lack of accuracy and may result in percentage errors up to ±10-15%. In many of the chemical methods an error of ±3-5% is to be expected, often because of interference by other substances.

It was a major consideration in the work reported here to develop a single method for the determination of a number of antibiotics.

To be a valuable addition to the present methods it must be rapid, simple, reliable and result in a degree of accuracy at least as good and preferably better than other methods now available.

Polarography has been used successfully in many fields of microassay. It was decided to explore the possibility of the application of the polarographic method to the measurement of antibiotics, both as chemically pure substances, and as ingredients in pharmaceutical preparations. An evaluation of the effects of the presence of other ingredients in the pharmaceutical preparations and the elimination of interfering substances was a part of this study.

Since it is of particular importance to measure the antibiotics in terms of its activity, rather than concentration alone, it was a further purpose of this work to determine the relationship between measurement of concentration by this method and the measurement of pharmacological activity by microbiological method and therefore to determine the effect of degradation of the active antibiotic on the measurement method.

II

LITERATURE SURVEY

Antibiotics have been measured for the purposes of quality control and dosage formulations by various methods. Microbiological methods are the recognized and approved methods for pharmaceutical analysis. Various chemical methods have been developed to increase the reliability of the measurements and have been used in place of the conventional microbiological methods provided they have shown good correlation with the latter methods with respect to pharmacological activity.

A. MICROBIOLOGICAL METHODS

The earliest methods of assaying antibiotics were the microbiological methods. The continuation of these as the recognized official methods is based on the fact that here the pharmacological activity is measured directly. All antibiotics can be measured by microbiological methods provided a suitable microorganism sensitive to the antibiotic is available. Grove and Randall have described various microbiological assay methods for individual antibiotics in their compilation of "Assay Methods of Antibiotics"(1).

The basis of all the microbiological assays is the simple comparison of inhibition of the growth of susceptible microorganisms produced by a known concentration of the standard preparation with that produced by a measured concentration of the sample being tested.

The commonest procedures in the microbiological methods include (i) Paper Disc Method, (ii) Cylinder-Plate Method, and (iii) Turbidimetric Method.

In the paper disc method circular filter paper discs are soaked with the antibiotic solution and then placed on the agar media, contained in a petri dish, which has already been seeded with a sensitive test organism. The antibiotic will diffuse into the media and inhibit the growth of the organism surrounding the paper disc.

The cylinder plate method (2-4) is exactly the same as the paper disc method except that the antibiotic solution is contained in open ended cylinders placed upright on the agar media. The solution diffuses out and produces the inhibition.

The turbidimetric method (5-7) is, on the other hand, based on the ability of the organism to grow in a culture broth in the presence of a certain concentration of an antibiotic. The degree of turbidity produced by an unknown concentration of the antibiotic is compared with that of a known concentration of the standard preparation in a suitable turbidimeter . The turbidity will increase with a decrease in concentration of the antibiotic or decreased effectiveness of the antibiotic.

The microbiological assay methods have undergone various modifications both in the technique as well as in method of calculation

in order to improve the efficiency, sensitivity, accuracy and reproducibility of the methods (8-12).

Kirshbaum and Arret (13) have compiled a table in which are listed the details required for some 36 different bioassay methods for antibiotics including a total of 17 of the most commonly used antibiotics.

It is obvious that the main advantages of the microbiological methods are their sensitivity and ability to directly measure the pharmacological activity of the antibiotic on a susceptible organism. Such methods are also usually insensitive to the presence of similar but pharmacologically inactive contaminants or degradation products.

There are, however, a number of serious disadvantages. Although, most sensitive, the method is too time consuming to be suitable for routine analysis in industry, and secondly, it fails in the case of a mixture of antibiotics such as are present in some pharmaceutical preparations, since the inhibitory effects will be additive for the individual components, and thus a previous separation is necessary. Although a number of modifications to increase the accuracy have been made, the variation between replicates is unduly large and a serious drawback. The percentage error for microbiological methods is ±10-15%, which is too large for satisfactory quantitative purposes. For these various reasons, the microbiological methods have been and are constantly being replaced wherever a reliable chemical method has been forthcoming.

B. CHEMICAL METHODS

Following the discovery of a new antibiotic, a microbiological test is normally the only immediately available method for its measurement. But as the structure of the antibiotic becomes known, methods based on its chemical properties begin to be developed.

There have been, as a result, numerous chemical methods applied to the assay of antibiotics. The success of any such method depends upon its ability to correlate well with the results obtained by microbiological methods. Since chemical methods are based on a reaction involving some portion or all of the molecular structure, it is always possible, indeed probable, that degradation products, which are generally inactive, may, because of only minor molecular changes, also give the same reaction and thus be measured together with the potent compound. Thus most chemical methods may give an inaccurate estimation of the amount of the active antibiotic present.

Grove and Randall have described chemical assay methods for a number of antibiotics in their monograph (1).

Colorimetry is probably the earliest chemical method applied to the determination of most of the antibiotics. This method, which is based on the production and measurement of some color produced by a reaction between some portion of the molecule and a specific reagent, generally suffers from simple interference from many substances containing similar functional groups in their molecules. To reduce

such interference spectrophotometric methods have been developed and with a fair degree of success. In some cases where antibiotics themselves produce fluorescence or do so after reacting with some reagents fluorimetry has found valuable applications.

Since most of these methods suffer seriously from interference when applied to formulations, interest has been focussed on developing chromatographic techniques for separation of the active principles prior to actual determination. Volumetric methods have also found some application in a number of titratable antibiotics but success has been limited because of various unavoidable factors in volumetric analysis. In a few cases (101) gravimetric methods of analysis have been employed with some success.

Because of the numerous chemical methods that have been developed and the many improvements to these methods which are constantly being made the survey of chemical methods will be limited to those most recently reported.

I. Tetracyclines

All the tetracyclines contain the same phenolic hydroxyl group and they all react with reagents like ferric chloride to give a measurable color (1). On heating with hydrochloric acid, chlorotetracycline and tetracycline both give a yellow color. Tetracycline and oxytetracycline also give the same yellow color on the addition of alkali. Chlorotetracycline under similar conditions gives a very unstable color (1).

Based on these properties there have been methods developed for the assay of all the tetracyclines. Monastero, Means, Grenfell and Hedger (14) early in 1951 developed a colorimetric method for the measurement of oxytetracycline based on the orange-brown color produced with ferric chloride. The same group of workers also developed a spectrophotometric method (14) based on the yellow color produced by oxytetracycline on treatment with alkali.

Yaskina and Filichkina (15) in 1962 described a modification of the ferric chloride method (14) in which ferrous chloride was used for the determination of chlorotetracycline HCl. Kerenyi (16) in 1958 reported another colorimetric method for oxytetracycline employing extraction with butanol followed by treatment with ferric nitrate. A new quantitative color test for differentiation of tetracycline, chlorotetracycline and oxytetracycline was developed by Terai (17), in which a solid sample or an evaporated residue of the test solution was treated with a few drops of cooled 97.0% sulphuric acid in a porcelain microcrucible. The presence of tetracycline, chlorotetracycline, or oxytetracycline is indicated by reddish-violet, indigo or rose-purple color respectively. Wesley-Hadzija and Fogec (18) used diazobenzene sulfonic acid to produce a yellow color with oxytetracycline. Murai (19) independently developed a method based on a reaction with indophenol which measured both tetracycline and chlorotetracycline in a mixture of these two.

Princeps (20), at about the same time, described a method giving five specific reactions for identification and simultaneous colorimetric measurement of all the tetracyclines. Uno and Morishita (21) utilized the chelate formation between diphenyl borinic acid and tetracycline, chlorotetracycline and oxytetracycline for colorimetric measurement of each of these antibiotics at different wave lengths. The determination of tetracycline and chlorotetracycline in a mixture was also done by Kolusheba and Koen (22). The method was a combination of the method for the determination of tetracycline as given in the USP (5) and the colorimetric method for chlorotetracycline reported by Levine, Garlock and Fischback (23).

Kakemi, Arita, Sezaki and Nadai (24) described another improved colorimetric method for the determination of tetracycline using p-nitro benzene diazonium chloride as the reagent. A colorimetric method reported by Bellotti and Mossini (97) for determining tetracycline in the presence of oleandomycin will be mentioned later (page 22).

Color developed in all the colorimetric methods is either completely pH dependent, not very stable or suffers from interference. Many of these methods require lengthy procedures of extraction, digestion, etc.

To reduce the somewhat uncertain nature of the colorimetric methods many workers have tried spectrophotometric methods. Chiccarelly,

Woolford and Avery (25) described in 1959 a spectrophotometric method for simultaneously determining tetracycline HCl and chlorotetracycline HCl using two different wave lengths.

Robert (26) developed another spectrophotometric method for measuring tetracycline, chlorotetracycline and oxytetracycline using procaine as the color reagent. Separate peaks occurred at three different wave lengths, one for each of the forms of tetracycline. Garrett (27) in the course of his investigation of the stability of tetracyclines, used a spectrophotometric method for the measurement of tetracyclines. Shah, Raman and Shah (28) reported in 1963 a spectrophotometric method for the determination of tetracyclines and chloramphenicol in a mixture, by measuring the extinction at different wave lengths.

The spectrophotometric methods are also only moderately sensitive and are subject to strong interferences from other colored substances.

Fluorimetric methods have been developed for all the tetracyclines and are based on the fluorescence emitted by the complex formed between the reagent and the antibiotic (29) or some product of degradation or hydrolysis of the antibiotic (30-33). As for example, the fluorimetric method for determining chlorotetracycline developed by Tomiyama, Yone and Kitahara (34), was based on the golden fluorescence emitted by this antibiotic in the chelate

form with potassium aluminum sulphate.

Murea and Cruceanu (35) have developed a simple, rapid and precise fluorimetric method for determining the same antibiotic by using zinc chloride as the reagent.

Yokoyama and Chatten (36) investigated the application of non-aqueous procedures which could be readily performed by visual titration for both the free base and hydrohalide salts of some of the tetracycline antibiotics. The method, however, must be modified where interference from other materials in formulations occurs and in some instances requires extraction of the antibiotic. These authors could not reproduce satisfactorily the earlier works of Ekeblad (37) and Sideri and Osol (38) who reported the determination of some tetracyclines in glacial acetic acid.

Recently a volumetric method has been employed for tetracycline measurement by Garrett (39) in the course of his pKa' value determinations.

A number of chromatographic methods have been developed (40-43) for the analysis of tetracyclines but they are primarily of value for identification and separation purposes prior to determination by other chemical methods already mentioned.

II. Streptomycins

The earliest colorimetric method for the measurement of streptomycin and dihydrostreptomycin is based on the conversion of streptomycin to maltol by heating with sodium hydroxide (1). Maltol can be measured as it is or can produce a color in a reaction with ferric chloride (44,45) or Folin and Ciocalteu phenol reagent (1). Dihydrostreptomycin gives maltol only when converted to streptomycin by boiling with sulphuric acid. Both streptomycin and dihydrostreptomycin, however, can be measured colorimetrically based upon a reaction of the guanidino group common to both (46). The main disadvantage of this method is that streptidine, which is a degradation product of both, gives the same color reaction.

Early in 1958 the groups of Natarajan and Tayal (47) and Barilari and Katz (48) described independently two colorimetric methods for measuring streptomycin and dihydrostreptomycin in mixtures. Agrawal and Dutta (49) described a similar colorimetric method in which they used picric acid as the reagent. Tetracycline, p-amino sulphonic acid, and isoniazid do, however, interfere in the color production.

Fontes (50) has advantageously used the modified Sakaguchi reaction (Weber, C.A. 24, 2485, 1930) by using 1-naphthol and sodium oxybromide for the determination of streptomycin and dihydrostreptomycin.

Kartseva, Chekulaeva, Korchagin and Burns (51) reported an improved maltol method for better results in the measurement of streptomycin in culture fluids. They absorbed the fluid on a column and subsequently eluted with solvent prior to the measurement of the antibiotic by the usual maltol method (44,45).

Machek (52) reported a procedure for measuring both streptomycin and penicillin in mixture. He used butyl acetate for extracting penicillin from the mixture. Streptomycin and penicillin then were measured by the standard methods. Shaw and Fortune (53) have reported the utilization of an autoanalyser based on the Ferric-Maltol-complex method (44,45) for the determination of streptomycin. Faure and Blanquet (54) in 1962 reported a method based on the fluorescence emitted by both streptomycin and dihydrostreptomycin in alkaline solution containing sodium 1,2-naphthoquinone-4-sulfonate for the assay of the two antibiotics. Guanine and such other derivatives as arginine, however, also fluoresce. The same group of workers (55) later on reported that the two antibiotics gave fluorescence at two different wave lengths when treated with sodium β-naphthoquinone-4-sulfonate allowing their identification and measurement.

Recently, Kiryakob (56) described an iodometric method for the determination of streptomycin.

Huettenrauch and Keiner (57) developed another volumetric method based on the titration of maltol produced by streptomycin on heating with sodium hydroxide. The titrant used was ceric sulphate.

III. Chloramphenicol

The earliest colorimetric method for measuring chloramphenicol was based on reducing the nitro group of the antibiotic to an amino group which can be diazotized and coupled with reagents like N-(1-naphthyl)ethylenediamine, to form a colored compound (58). Later on, Ito and Okamoto (59) improved this method for the microdetermination of this antibiotic by modifying the coupling reagent. Barilari and Katz (60) described a colorimetric method for assaying chloramphenicol based on the determination of formaldehyde liberated by acid hydrolysis of the antibiotic. Two Japanese workers, Kakemi and Ohashi (61) almost at the same time reported another colorimetric method for the determination of this antibiotic using isoniazid as the reagent. The method involved various steps of extraction and centrifugation.

Another Japanese scientist, Yoneda (62) early in 1957 developed an ultra-violet spectrophotometric method for the measurement of chloramphenicol in otic preparations.

Simultaneous determination of chloramphenicol and prednisolone present in an ointment was reported by Yokoyama and Ito (63). Cyclohexane and ethanol were added to give a suspension and centrifugation of the suspension resulted in a sample which gave good results. The same

procedure was found satisfactory for ointments containing chloramphenicol and neomycin.

Lorenz and Machek (64) developed a spectrophotometric method for chloramphenicol in a preparation containing a sulfonamide based on the simple measurement of extinction of an ethanolic solution of the sample and subsequent correction by subtraction of the absorption due to the presence of the sulfonamide which was determined by the Bratton-Marshall method (65).

Halot (66) reported in 1961 the use of absorption spectrophotometry in the ultra-violet region for the determination of chloramphenicol preparations. He used different conditions for separating the active principle from the excipients in the various pharmaceutical forms such as suppositories, ointments and solutions.

The spectrophotometric method of Shah et al.(28) for determining chloramphenicol and tetracycline in mixture has already been mentioned.

Hughes and Diamond (67) very recently published a simple chemical method for the measurement of chloramphenicol in blood using isonicotinic acid as the reagent for color production.

A few volumetric methods have also been reported for chloramphenicol measurements. The first efficient volumetric method was that of Franchi and Pellerano (68) in which the excess of propionic anhydride

remaining after esterification of the antibiotic with this compound was titrated. The method, it was suggested, could also be used for pharmaceutical preparations of this antibiotic. Srivastava, Ray and Mukerji (69) at about the same time put forward another volumetric method for chloramphenicol measurement. They determined the amount of the antibiotic from the amount of sodium nitrite required to diazotize the amino form of chloramphenicol.

IV. Neomycin Sulphate

Bulant and Parizkova (70) in 1962 described a spectrophotometric method for the determination of neomycin base and salts using ninhydrin as the color reagent. Furfural, however, gave the same color reaction and caused interference.

Morgan, Honig, Warren and Levine (71) used a combination of chromatography and spectrophotometry for the assay of neomycin in pharmaceutical preparations. The method consisted in the hydrolysis of the antibiotic to furfural, the purification of the furfural by column chromatography and subsequent determination by colorimetry using p-bromoaniline.

Pan and Dutcher (72) early in 1956 reported a paper chromatographic method for separation of acetylated neomycins which was also semiquantitative.

In 1963, Brodasky (73) introduced thin layer chromatography on carbon plates for the separation and quantitation of the different neomycin sulphates by a microbiological method using Bacillus pumilus. Very recently, Foppiano and Brown (74) have shown that both qualitative and quantitative determinations can be performed by thin layer chromatography, using ninhydrin for identification. For quantitative measurement the neomycin zones are removed and the ribose moiety of the antibiotic is determined colorimetrically using orcinol-ferric chloride-hydrochloric acid-acetic acid reagent as employed by Tomada (75) and Korchagin, Korobitskaya, Druzhinina and Semenov (76).

The method of Yokoyama and Ito (63) for measurement of chloramphenicol and neomycin in ointment mixture has already been mentioned.

V. Erythromycins

Korchagin and co-workers (77) developed in 1961 a colorimetric method for the determination of erythromycin by measuring the optical density after heating the antibiotic with sulphuric acid. But the main disadvantage of the method was that it also measured the inactivation product of erythromycin. Kuzel, Woodside, Comer and Kennedy (78) described a spectrophotometric method for the determination of erythromycin based on increased absorption after mild alkaline hydrolysis of the antibiotic. Ermakova, Korchagin, Vakulenko and Sidorova (79) developed a conductometric titration procedure for the measurement of this antibiotic. This method suffered from interference by the inactivation

products obtained during hydrolysis which was involved in the procedure. The identification reaction of erythromycin lactobionate developed by Unterman (80) could also be used for quantitative measurement, using citric acid and magnesium gluconate for color production.

Ochab, Malysz and Borowiecka (81) developed a chromatographic method for the separation of erythromycin mixed with tetracycline prior to its determination spectrophotometrically using xanthydrol for the final color production.

The chromatographic methods, including thin layer chromatography (82), are all for the purposes of separation and qualitative determination.

VI. Penicillins

One of the earliest chemical methods for the determination of penicillin was the iodometric method of Alicino (83). The method, which consisted of decomposing the active antibiotic into an inactive product, penicilloate, suffered seriously from interference in the case of formulations where starch or sugars were present. Another chemical method for the determination of penicillin is based on its conversion to hydroxamic acid which forms a colored complex with ferric chloride (1). Carta-De Angeli, Ventura and Di Accadia (84) devised a simplified method based on this latter property of penicillin for the determination of the antibiotic in salts and fermentation media. It has been found, however, that the reaction, although complete, was not

specific, since many esters, anhydrides and amides also reacted with the reagent to form hydroxamic acid (85).

Nogami and Kanazawa (86) proposed that phenoxymethyl penicillin (penicillin v) could be measured by coupling it with methyl green. A year later, Desphande and Karmarkar (87) utilized phosphomolybdic acid as the color reagent in the colorimetric determination of procaine penicillin G. Unterman (88) gave another colorimetric method for determining procaine penicillin in the presence of other antibiotics using p-dimethyl aminobenzaldehyde as the color reagent. Streptomycin and the potassium salt of benzyl penicillin did not interfere.

In 1962 Fursov (89) used colorimetry for the determination of penicillin V with an error of ±4%, which was claimed to be better than the iodometric method.

Very recently there has been a report of another colorimetric method for determination of procaine penicillin in mixed feeds by Stone (90) in which penicillin is converted to penicillamine which turns blue with arsenomolybdic acid reagent.

Weaver and Reschke (91) described an ultra-violet spectrophotometric method for the determination of methicillin, a synthetic penicillin, based on the fact that methicillin when heated in acetate buffer showed an absorption maximum at 330 mμ.

Yakobson and Ermolova (92) used nephelometry for the stability measurement of penicillin N,N-dibenzylethylenediamine (bacillin I).

Kurzawa and Suszka (93) developed a volumetric method with an average error of ±5% for the determination of penicillin G or its procaine salt in the presence of streptomycin and chlorotetracycline. Reference has already been made to the application of thin layer chromatography by Nicolaus et al. (42) for the detection and determination of penicillin and other antibiotics.

There have been other chromatographic methods (94-96) both on paper and thin layer chromatography but they are of principal value for the separation and purification of different penicillins and therefore are of significance only for qualitative purposes.

VII. Oleandomycin

There has not been much reported on the determination of oleandomycin. Bellotti and Mossini (97) in 1960 described a good but lengthy colorimetric method for determining oleandomycin in the presence of tetracycline. Tetracycline could also be measured by this method by measuring the absorbance at a different wave length than that for oleandomycin and under a different condition.

Fukuzawa and Shiizu (98) employed ultra-violet spectrophotometry for the determination of oleandomycin based on destroying the antibiotic by alkali treatment and subsequent measurement of some degradation product.

Lees (99) described a paper chromatographic method for the separation of this antibiotic from multicomponent antibiotic preparations.

VIII and IX. Bacitracin and Polymyxin

Agrawal and Dutta have published a review article (100) on the methods of analysis of bacitracin and many other antibiotics.

Maturana, Dannier and Brieva (101) in 1962 described some 13 color and precipitation reactions for the detection and several color and precipitation reactions for the determination of polypeptide antibiotics including bacitracin and polymyxin. Precipitation reactions were not suitable for colorimetric determination of these antibiotics, but gravimetric determination of bacitracin and polymyxin was possible based on the precipitation reaction of these antibiotics with phosphomolybdic acid.

In summary, although chemical methods allow a fair degree of accuracy, they suffer from a variety of disadvantages. Prominent among these is interference and in an effort to remove the sources of interference, lengthy procedures of extraction and separation by chromatography and other means must be employed. Moreover, almost all of the chemical methods are quite lengthy procedures themselves, involving a number of steps and considerable care in sample preparation. In addition, a different chemical method must normally be used for the different antibiotics, thus requiring a series of reactions under different experimental conditions, standard graphs, as well as a variety of analytical instruments.

III

METHODS

A. Polarography

The polarographic method of analysis was invented by Jaroslav Heyrovsky (102). It is based on the unique characterestics of the current-voltage curves obtained during electrolysis. For most polarography a cell is used in which one electrode is a dropping mercury electrode. The mercury is discharged in the form of a definite sized drop and at a constant rate from a fine bore capillary glass tube. Under appropriate conditions, when a definite potential is applied between the two electrodes in the polarographic cell, a typical current-voltage curve is produced as shown in Figure 1. Given the conditions of the measurement it is generally possible to identify the presence of a particular sub-stance as a result of the position of the half-wave potential (E 1/2). In addition, the extent of the diffusion current, as shown in Figure I, may be used as a measure of the concentration of the substance.

The theoretical principles of polarography are beyond the scope of this presentation but have been discussed at length in a number of texts (103,104).

Ilkovic (105) examined the various factors governing the diffusion current and deduced the equation for diffusion current which was further confirmed by MacGillavry and Rideal (106),

$id = 607nD^{1/2} C m^{2/3} t^{1/6}$, where

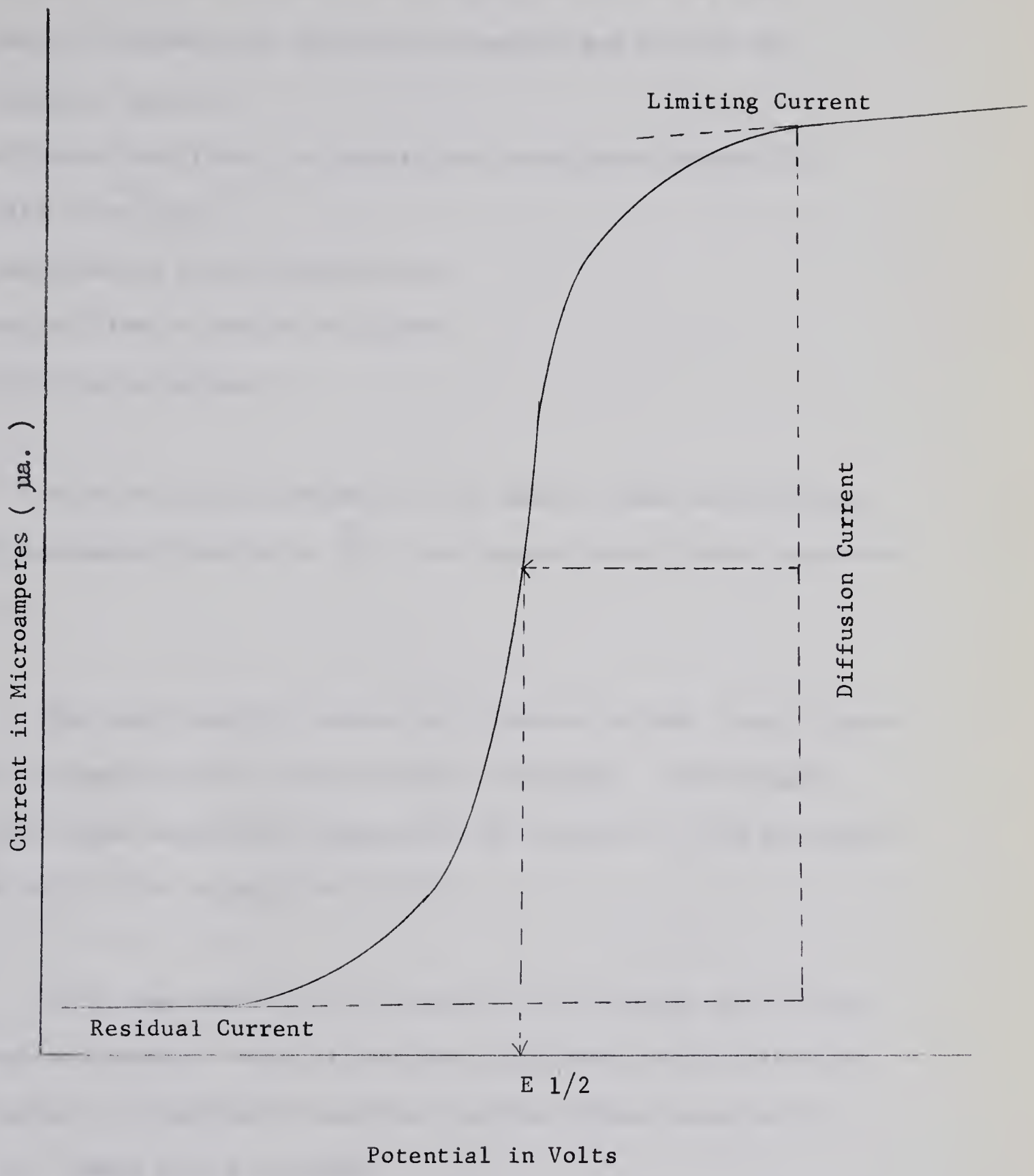


Figure 1. The normal current-voltage wave



id = average diffusion current in μa during the life of a drop.

n = number of Faradays of electricity required per mole of the electrode reaction.

D = diffusion coefficient of reducible or oxidisable substance in units of $cm^2\ sec^{-1}$.

C = concentration in millimoles/litre.

m = rate of flow of mercury in mg/sec.

t = drop time in seconds.

From the Ilkovic equation it is apparent that the diffusion current increases directly as $\sqrt[6]{t}$, and varies directly with concentration, C.

The drop time (t), varies as a function of EMF, first increasing with potential up to -0.52v and then decreasing. The diffusion coefficient also varies with temperature at the rate of 1-2% per degree rise or fall in the vicinity of $25.0^{o}C$.

Drop time should be in the order of 2-5 seconds but in any case must be constant. When all the factors except concentration are kept constant, an important deduction from the Ilkovic equation is,

$id = k.c$, where k is a constant;

i.e. diffusion current is directly proportional to the concentration of the electroactive substance. This is the basis of quantitative analysis by the polarographic method.

The polarographic method of analysis is very suitable for inorganic analysis since so many elements and inorganic compounds are reduced at the dropping mercury electrode giving well defined polarograms. A few current references only are included (107-114). Accordingly, this method has found very wide application in the field of metallurgical analysis for determining component parts or impurities of alloys (115,116). It also has found wide application in the determination of trace metallic impurities in commercial chemicals, or trace inorganic constituents of a host of materials ranging from waters and petrol, to various organic and biological materials and foodstuffs (117-121).

A great variety of organic compounds, including various aldehydes and ketones, unsaturated acids, nitro and nitroso compounds, azo and diazo compounds, halogenated compounds, quinones of various types, peroxides and many others are reducible at the dropping mercury electrode (122-125). The determination of oxygen in gases and solutions is another uniquely valuable application of polarographic analysis (126,127). The greatest percentage of papers devoted to applied organic polarography describe applications to the problems of pharmaceutical analysis (128). Fields where polarography has been increasingly used include agriculture and food chemistry, plastics, textile industry, etc. (123).

Aside from practical analysis, polarography provides a fruitful means of studying oxidation-reduction processes, reaction rates and equilibria, organic synthesis of natural products and analysis of the structures of organic substances (123).

Polarographic analysis is not limited to aqueous solutions, and can be applied to solutions in certain nonaqueous solvents (117, 118,146). The essential condition is that the solvent be sufficiently polar. The use of non-aqueous solvents is particularly useful with many water-insoluble organic substances (117,118,146). It is even possible to obtain polarographic waves in molten salt media.

The polarographic method of analysis is highly sensitive and accurate. It can measure concentration in the range of 10^{-3} - 10^{-5} M and in some cases up to 10^{-6} M with maximum average error of ± 2.5%. The method needs only a small volume of solution for analysis and the active material can also be recovered if so desired. It is possible to detect and determine simultaneously more than one substance from the $E_{1/2}$ values and diffusion currents. The last, and indeed one of the most important advantages of polarographic analysis is its speed. It is possible to do up to fifteen determinations per hour in routine analysis.

B. Evaluation of Quantitative Results

There are various ways of evaluating the unknown concentration of any substance by polarographic analysis among which the following are the more common:

(i) Method of standard graph:

This is the conventional method and is based on the construction of a standard graph on ordinary graph paper by plotting diffusion current against concentration. The unknown concentration is determined from the standard graph by determination of its diffusion current.

(ii) Method of standard addition (129):

In this method the diffusion current of the unknown solution is determined first and then a known volume of the standard solution is added to the unknown solution and the diffusion current of the total solution is measured. The unknown concentration is then calculated from the equation

$$x = c \frac{n\ id_2}{id_2(m + n) - mid_1}$$, where

x = concentration of the unknown/ml. of test solution

id_1 = diffusion current of the unknown

id_2 = diffusion current of the unknown plus the standard

m = ml. of the test solution

n = ml. of the standard solution added

c = concentration of the standard/ml. of the standard solution

(iii) Direct comparison:

This is the most accurate method for evaluating unknown concentration with the help of a standard graph. Although the standard graph is a good method for determining an unknown, it is somewhat

inconvenient for routine analysis. A standard must be prepared daily or checked at various points on the straight line. In polarographic analysis, however, there is usually some slight variation from day to day due to various factors already mentioned (105). For this reason direct comparison is most convenient and the unknown and the standard under similar conditions of pH etc. should give similar waves at the same E 1/2. The method consists in first measuring the diffusion current for the test solution. Then the concentration of the standard is chosen with the help of a standard graph in such a way that under similar conditions of measurement it gives a diffusion current as close to that for the unknown as possible. The concentration of the unknown is then determined from the simple relation

$$x = c \frac{id_2}{id_1}, \text{ where}$$

c = concentration of the standard

x = concentration of the unknown in the same unit as for the standard

id_1 = diffusion current for the unknown

id_2 = diffusion current for the standard

id_1 and id_2 are very nearly equal.

(iv) Absolute method:

This is of course theoretically the best method provided all the factors of the Ilkovic equation (105) except concentration are known accurately. The diffusion current is measured from the

polarographic wave obtained by electrolysing the test solution and the concentration in millimoles per litre is calculated from the equation (105).

C. General Procedure in the Polarographic Analysis

The application of polarography to the estimation of antibiotics required in this work was very simple. A certain amount of the antibiotic powder, either as the chemically pure material* or as a pharmaceutical preparation was weighed on an analytical balance. The powder was dissolved in the appropriate aqueous solvent and the volume made up to the mark in a volumetric flask. In the case of tablets, weights of ten tablets were taken individually and the mean was determined. A few tablets were finely powdered in a mortar. The capsules generally contained the material in a powder form. When the pharmaceutical preparation was in the form of a solution or syrup, dilution was the only step necessary to prepare a working stock solution. To obtain clear solutions of pharmaceutical preparations 0.1 to 0.2 ml of 3M HCl, and in a few cases up to 0.25 ml of a suitable additional solvent, was added to assure complete solution of the antibiotic. The presence of various other ions sometimes interfered with the free solubility of the antibiotic in water.

From the stock solution, a known volume was pipetted out into a volumetric flask of appropriate size, and a suitable volume of supporting electrolyte and/or buffer was added. When necessary a

*The chemically pure material was in most instances USP reference standard. In all other instances pure compound was obtained from pharmaceutical houses and was stated to have a potency based on the USP reference standard.

maximum suppressor, generally gelatin, was added and the volume made up to the mark. The pH of this solution was checked and if necessary adjusted to the desired level and then it was poured into the electrolysis cell. Dissolved oxygen was removed by bubbling pure nitrogen through the solution for a sufficient time. The sample was then electrolysed through the desired potential range with an automatic recording being made of the polarogram. The E 1/2 and the diffusion current were measured from the polarogram. A Leeds and Northrup's Electrochemograph G was the instrument employed in the work. The capillary constant, $m^{2/3}t^{1/6}$ can be determined from the mass of mercury flowing per second and the drop time according to ii and iii below.

The different factors of the Ilkovic equation during the whole period of this work were as follows:

(i) Temperature - The range of temperature was from 23 to 27°C during the entire course of the work. The variation during any set of experiments was checked within ±0.5°C with the help of the water bath surrounding the cell.

(ii) Drop time (t):-'t' varied in the range of 3 to 4.5 seconds with variation between replicates in the same set of experiments being controlled to ± 1%.

(iii) Mass (m) of mercury flowing per second :- 'm' was fairly constant throughout the use of the same capillary within the range of 1.45 to 1.55 mg/sec at various E 1/2 values for different antibiotics, but the variation of 'm' between replicates was kept at ±0.1%.

(iv) Deaeration time :- Passing nitrogen at the rate of 7-10 bubbles/second for 3 - 5 minutes was sufficient to remove all

the oxygen present in solution.

(v) pH :- pH readings for replicates were checked to ±0.05 of a unit.

(vi) Base electrolyte or buffer concentration :- This was always about 100 times higher than the highest concentration of the substance being analyzed.

D. Microbiological Comparison

Any chemical method of analysing antibiotics, to be valid and acceptable, must agree completely with the official microbiological method which measures the pharmacological activity of the antibiotics or a satisfactory correction factor must be determined.

For the polarographic method to be particularly useful, it must not measure anything other than the pharmacologically active form of the antibiotic. The possibility existed that a serious shortcoming of the polarographic method would be that it might measure under the established conditions the inactive or degradation products obtained by deterioration of antibiotic pharmaceuticals together with the active components. Under ordinary conditions it has been found that the preparations undergo detectable amounts of deterioration over a long period of time. In order to evaluate this effect, the antibiotics were heated for sixteen hours at different temperatures and comparative assays were carried out of the quantity of the antibiotic remaining according to the polarographic method and the bacteriocidal activity remaining according to the microbiological method. The diffusion current obtained in each case and the diameter of the zone of inhibition

were plotted for the same antibiotic on the same graph against the temperature used. For the microbiological assays, the cylinder plate method (2-4) was employed using Bacillus subtilis as the test organism. The incubation period was twenty hours at $30^{o}C$.

The correlation coefficient (r) between the diffusion current and the diameter of the zone of inhibition at different temperatures for the individual antibiotics was calculated according to the formula (130)

$$r = \frac{\Sigma XY - \frac{\Sigma X \Sigma Y}{n}}{\sqrt{\left[\left(\Sigma X^2 - \frac{(\Sigma X)^2}{n}\right)\left(\Sigma Y^2 - \frac{(\Sigma Y)^2}{n}\right)\right]}}$$

where, X = diffusion current

Y = diameter of the zone of inhibition

n = number of sets of readings

There was only one independent variable, namely, temperature.

IV

EXPERIMENTAL AND RESULTS

I. Tetracyclines

All the important members of the tetracyclines were found to be measured satisfactorily by the polarographic method.

(i) Tetracycline Hydrochloride

Polarographyhas not previously been applied to the measurement of tetracycline HCl. Fait (131) in 1963 reported an oscillographic method for the identification of tetracycline, Cl-tetracycline and oxy-tetracycline. A recent reference was obtained after the work was completed in which the HCl of tetracycline had been examined polarographically (131a).

Using the polarographic method, in the present work, it was found that tetracycline HCl gave three imperfect or poorly developed waves through acid pH's and up to a pH of 7.35. From that point upwards there appeared two well developed waves until about pH 9.00, beyond which both the waves began to lose their typical shape.

Quantitative measurements could be made with a high degree of accuracy in the pH range of 7.00 to 9.00, and pH 7.75 was found to be optimum for shape and sensitivity (Figure 2).

Both phosphate and borate buffer could be used to control the pH to within ± 0.05 of a unit. If phosphate buffer was used, however, at concentration higher than 2.00 mg. tetracycline HCl/10 ml. there appeared a maximum at the first wave and although gelatin could correct the situation, it also made the resolution poor. It was found necessary

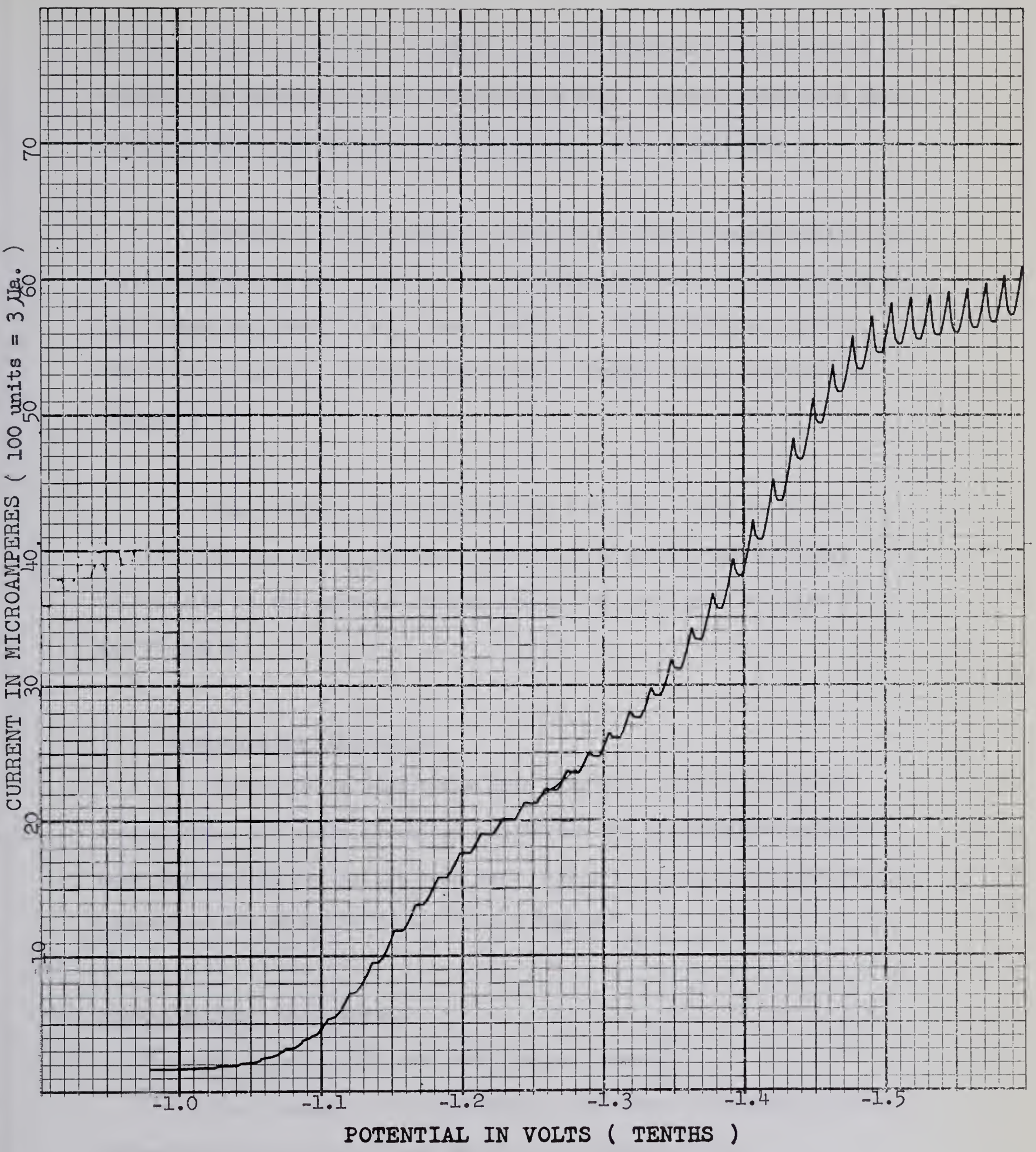


FIGURE 2. THE CURRENT-VOLTAGE WAVE OF TETRACYCLINE HYDROCHLORIDE



to use different amounts of gelatin for each different concentration to suppress the maximum without removing the peak. Borate buffer was thus preferred since the addition of gelatin was found unnecessary.

Because of the residual current, the lowest concentration that could be measured was 5 μg/ml. and the diffusion current values from the first wave were used in all cases (Table 1). The diffusion current when plotted against concentration gave a straight line relationship (Figure 3).

The half-wave potentials at pH 7.75 were found to be -1.17 ± 0.01 v for the first wave and 1.40 ± 0.01 v for the second wave. The results of the microbiological comparison are shown in Table II and Figure 4.

Tetracycline Amphoteric

Tetracycline amphoteric gave identical waves to those of tetracycline HCl under identical conditions and quantitative measurement was carried out in the same way. The results of the microbiological comparison are shown in Table III and Figure 5.

(i)a. Pharmaceutical Preparations

1. Tetracyn Capsules (Pfizer Corporation, Canada)

Each capsule of the preparation is stated to contain 250 mg. tetracycline HCl and the same amount of glucosamine HCl.

Table I

Data on the Diffusion Currents of the First Wave of Tetracycline HCI in Borate Buffer at pH 7.75 ± 0.05. $m^{2/3} t^{1/6} = 1.69$ mg. $^{2/3}$ Sec. $^{-1/2}$

Concentration mg/10 ml.	Diffusion Current 100 Units = 3 µa.	Average ± S.D.M.
0.05	2.38 2.25 2.25 2.38 2.25	2.3 ± 0.1
0.125	4.50 4.75 4.75 4.75 4.75	4.7 ± 0.1
0.25	9.00 9.25 9.00 8.75 9.00	9.0 ± 0.2
0.375	13.25 13.00 13.00 13.25 12.75	13.0 ± 0.2
0.50	17.50 17.00 17.75 17.25 17.50	17.4 ± 0.3
0.75	25.25 26.25 25.00 25.50 25.75	25.6 ± 0.5
1.00	34.50 34.00 34.25 35.00 33.50	34.2 ± 0.6
1.25	42.00 42.75 41.25 43.00 41.75	42.2 ± 0.7

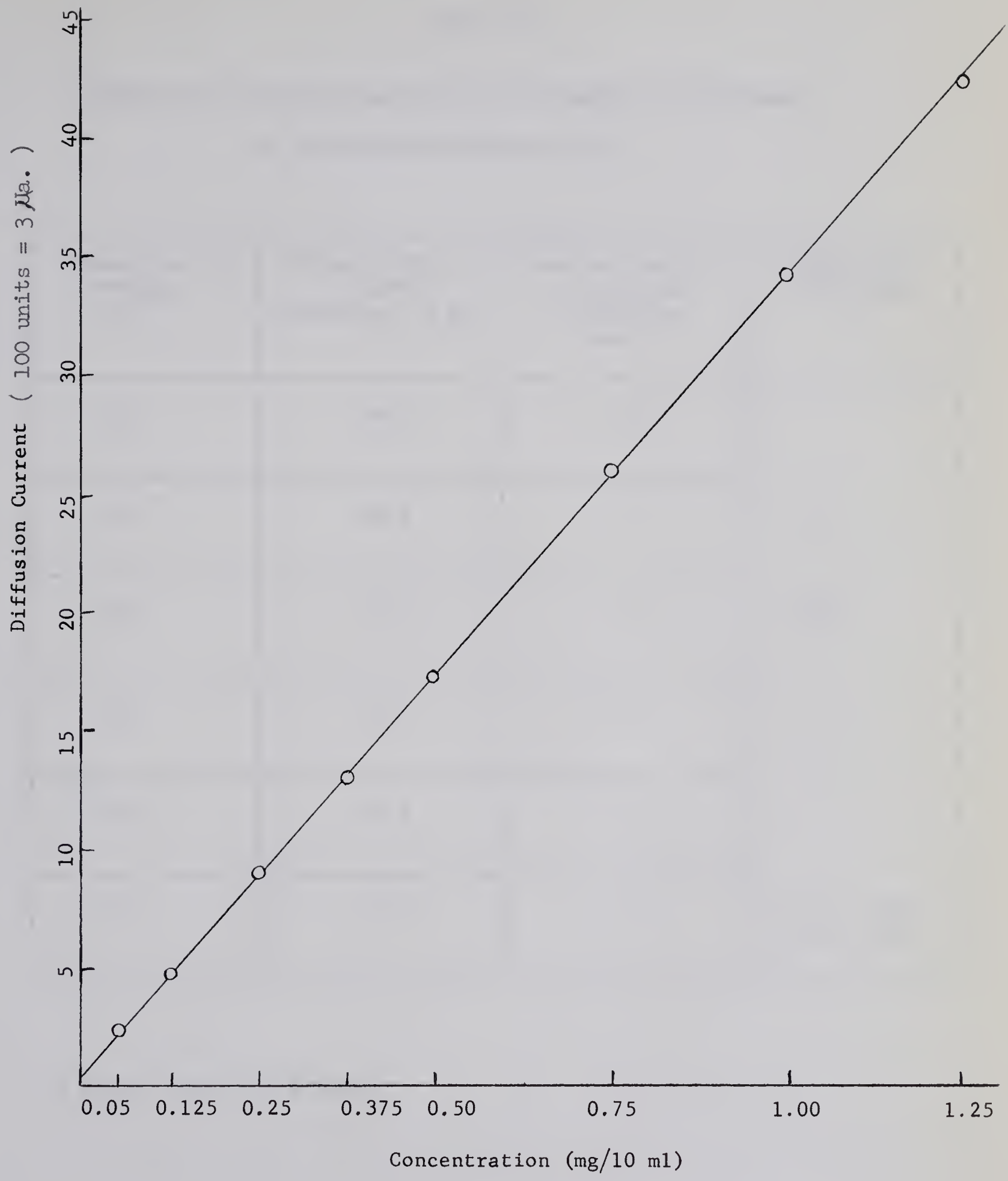


Figure 3. The graph of Tetracycline Hydrochloride standard.



Table II

Comparison of Microbiological and Polarographic Measurement of Tetracycline Hydrochloride

Temperature of Heating 0° C	Average Diffusion Current 100 units = 3 Ma.	Average Diameter of zone of inhibition mm.	Correlation Coefficient r
100	24.2	19	
110	24.2	19	
120	21.0	18	0.97*
130	16.0	16	
140	8.5	12	
150	1.8	4	95% = 0.878 99% = 0.959

* Significant at 99% level

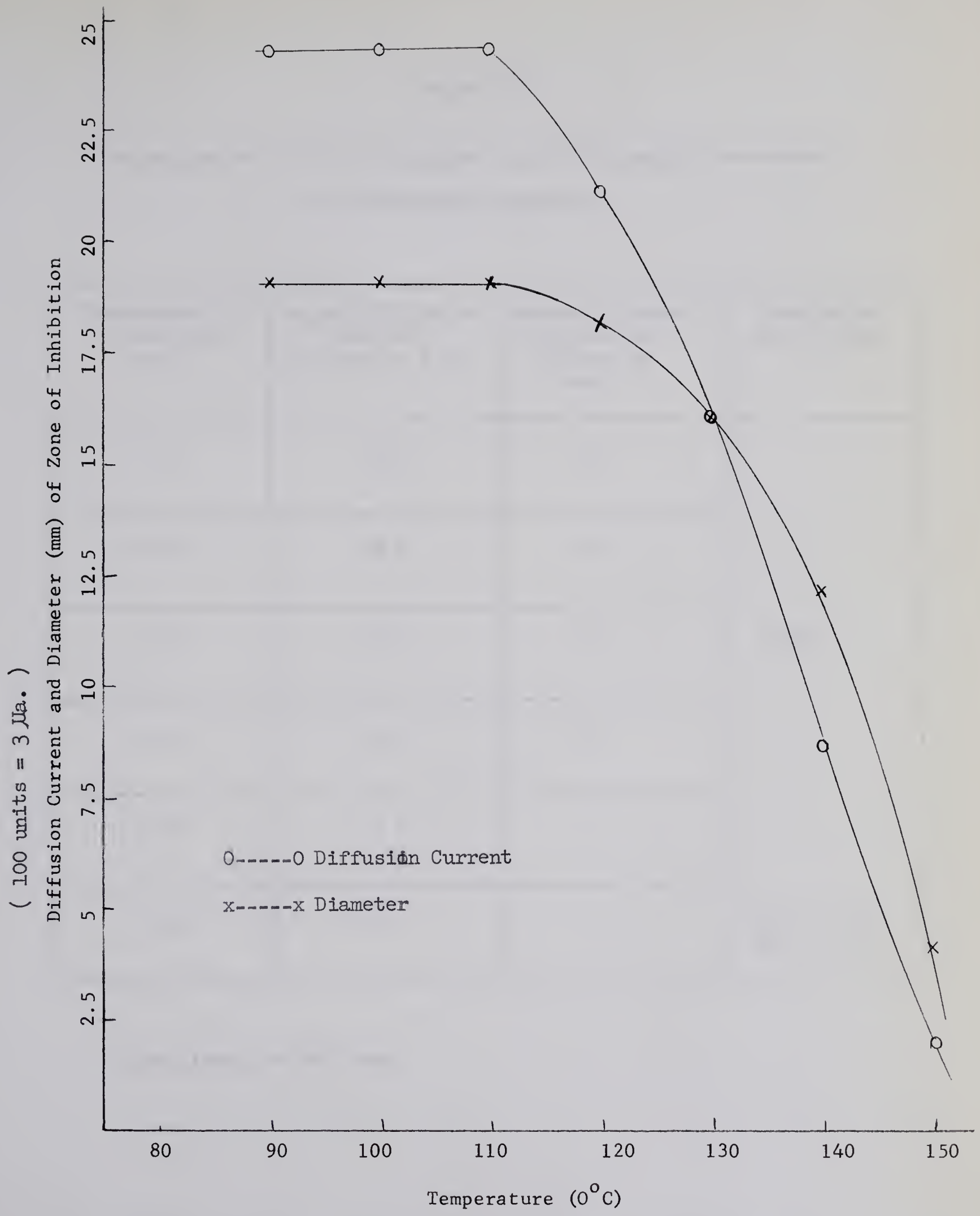


Figure 4. The comparison of microbiological and polarographic measurement of Tetracycline HCl during degradation.

Table III

Comparison of the Microbiological and Polarographic Measurement of Tetracycline Amphoteric.

Temperature of heating $0^{o}C$	Average Diffusion current 100 units = 3 µa.	Average Diameter of zone of inhibition mm.	Correlation Coefficient r
90	23.0	18	
100	23.0	18	
110	21.5	18	0.99*
115	17.0	14	
120	7.0	1	
130	0	0	95% = 0.95 99% = 0.99

* Significant at 99% level.



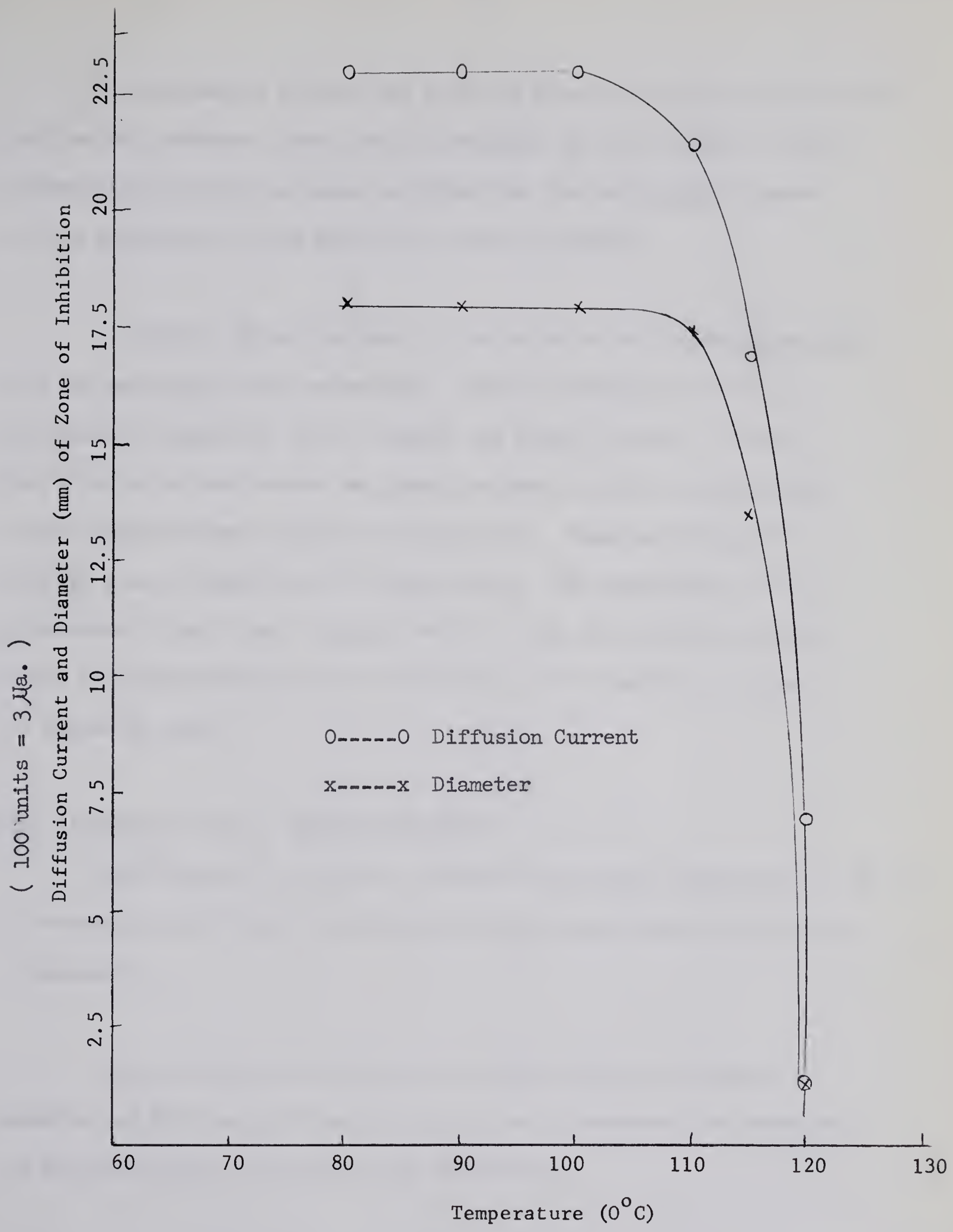


Figure 5. The comparison of microbiological and polarographic measurement of Tetracycline Amphoteric during degradation.

Glucosamine HCl did not give any wave in the region where tetracycline HCl produced a wave and the addition of this compound to pure tetracycline HCl did not have any effect on the polarographic waves of the antibiotic or the diffusion current obtained.

Weights of the contents of ten capsules were taken separately and the mean weight was determined. Both the tetracycline HCl and glucosamine components of the capsule are highly soluble in water. The solution of the powder was therefore made in water leaving some other components and excipients undissolved. These were found insoluble even in highly acid or basic media. The suspension or the supernatant liquid thus obtained could be used for analysis and was found to give essentially the same results. The results are shown in Tables IV and V.

2. Signemycin Capsule (Roerig, Canada).

Each capsule is stated to contain tetracycline equivalent to 167 mg. of tetracycline HCl and oleandomycin phosphate equivalent to 83 mg. of oleandomycin.

Since tetracycline base is not highly soluble in water the addition of HCl (as specified in the section on methods) was necessary to bring about the solution of the antibiotic.

The standard tetracycline waves at approximately -1.17 v and -1.40 v were easily distinguishable from that of oleandomycin phosphate which

Table IV

Results on the Polarographic Determination of the Concentration of Tetracycline Hydrochloride in Tetracyn Capsule (Pfizer - 250 mg./capsule)

Amount of the preparation mg./10 ml.	Tetracycline HCl found mg./10 ml.	Tetracycline HCl/capsule mg.	% Stated Concentration
0.50	0.197 ± 0.006	266.1 ± 7.7	106.4 ± 3.1
0.75	0.297 ± 0.006	267.2 ± 5.4	106.9 ± 2.2
1.00	0.400 ± 0.008	269.8 ± 5.4	107.9 ± 2.2
1.50	0.600 ± 0.011	269.8 ± 5.1	107.9 ± 2.0
2.00	0.791 ± 0.017	266.8 ± 5.6	106.7 ± 2.2

Average =
107.2 ± 0.7

Individual experimental results are presented in Appendix I, Table I.

Each result is an average of five determinations ± Standard Deviation of the Mean.



Table V

Results on the Polarographic Determination of the Recovery of Added Tetracycline Hydrochloride from Tetracyn Capsule.

Amount of the preparation mg./10 ml.	Tetracycline HCl (99.0%)				
	Amount added mg.	Total present mg./10 ml.	Total found mg./10 ml.	Amount recovered mg.	% Recovery
0.75 mg. = 0.297 mg. Tetracycline HCl = 0.300 mg. Tetracycline HCl (as 99.0%)	0.125	0.425	0.425 ± 0.003	0.123 ± 0.003	98.6 ± 2.6
	0.250	0.550	0.550 ± 0.005	0.250 ± 0.005	100.0 ± 2.1
	0.375	0.675	0.672 ± 0.005	0.371 ± 0.005	99.0 ± 1.4
	0.500	0.800	0.798 ± 0.012	0.498 ± 0.012	99.5 ± 2.4
	0.750	1.050	1.046 ± 0.012	0.745 ± 0.012	99.4 ± 1.6

Average = 99.5 ± 0.4

Individual experimental results are presented in Appendix II, Table I.
Each result is an average of five determinations ± Standard Deviation of the Mean.



appeared at -1.76 v at pH 7.75. This pH was used for the analysis of both antibiotics. Oleandomycin should not, therefore, interfere with the two waves of tetracycline and in fact it did not as was proved by the addition of oleandomycin phosphate to pure tetracycline HCl. The analysis was carried out using the suspension of the powder. Those preparations where insoluble excipients were present or preparations which were suspensions could be determined with equal facility to clear solutions. The results in tabular form are shown in Tables VI. and VII.

3. Tetrosol (F.W. Horner Limited, Canada)

Each buffered tablet is stated to contain 250 mg. tetracycline HCl. The tablets have a hard brittle coating which it was found difficult to prepare in a powder form.

Five tablets were weighed separately and the mean weight was determined. They were finely powdered in a mortar and a known weight was taken and water added. The aqueous solution was not clear due to the presence of some of the insoluble excipients and pieces of the coating. Addition of acid in the specified amount dissolved the particles of the coating. This stock solution was used for analysis. The results are shown in TablesVIII and IX.

Table VI

Results on the Polarographic Determination of the Concentration of Tetracycline Hydrochloride in Signemycin Capsule (Roerig - 167 mg./capsule)

Amount of the preparation mg./10 ml	Tetracycline HCl found mg./10 ml.	Tetracycline HCl/capsule mg.	% Stated Concentration
0.25	0.109 ± 0.003	175.7 ± 4.5	105.2 ± 2.7
0.50	0.216 ± 0.006	174.6 ± 4.6	104.5 ± 2.8
1.00	0.431 ± 0.009	173.9 ± 3.8	104.1 ± 2.3
1.50	0.648 ± 0.014	174.0 ± 3.7	104.2 ± 2.2
2.00	0.863 ± 0.023	173.9 ± 4.6	104.1 ± 2.7

Average =

104.4 ± 0.5

Individual experimental results are presented in Appendix I, Table II.

Each result is an average of five determinations ± Standard Deviation of the Mean.

Table VII

Results on the Polarographic Determination of the Recovery of Added Tetracycline Hydrochloride from Signemycin Capsule

Amount of the preparation mg./10 ml.	Tetracycline HCl (99.0%)				
	Amount added mg.	Total present mg./10 ml.	Total found mg./10 ml.	Amount recovered mg.	% Recovery
0.625 mg. = 0.270 mg. Tetracycline HCl = 0.273 mg Tetracycline HCl (as 99.0%)	0.125	0.398	0.399 ± 0.003	0.126 ± 0.003	101.0 ± 2.5
	0.250	0.523	0.524 ± 0.006	0.251 ± 0.006	100.5 ± 2.4
	0.375	0.648	0.650 ± 0.006	0.376 ± 0.006	100.4 ± 1.6
	0.500	0.773	0.774 ± 0.012	0.501 ± 0.012	100.2 ± 2.3
	0.750	1.023	1.026 ± 0.016	0.753 ± 0.016	100.3 ± 2.1

Average = 100.5

Individual experimental results are presented in Appendix II, Table II.

Each result is an average of five determinations ± Standard Deviation of the Mean.



Table VIII

Results on the Polarographic Determination of the Concentration of Tetracycline Hydrochloride in Tetrosol (F.W. Horner - 250 mg./tablet)

Amount of the preparation mg./10 ml.	Tetracycline HCl found mg./10 ml.	Tetracycline HCl/Tablet mg.	% Stated Concentration
0.50	0.236 ± 0.004	245.3 ± 3.8	98.1 ± 1.5
0.75	0.352 ± 0.006	243.9 ± 4.4	97.5 ± 1.7
1.00	0.467 ± 0.011	242.4 ± 5.8	96.9 ± 2.3
1.50	0.703 ± 0.016	243.3 ± 5.5	97.3 ± 2.2
2.00	0.939 ± 0.020	243.7 ± 5.2	97.5 ± 2.1

Average = 97.5

Individual experimental results are presented in Appendix I, Table III.

Each result is an average of five determinations ± Standard Deviation of the Mean.

Table IX

Results on the Polarographic Determination of the Recovery of Added Tetracycline Hydrochloride from Tetrosol.

Amount of the preparation mg/10 ml.	Tetracycline HCl (99.0%)				
	Amount added mg.	Total present mg/10 ml.	Total found mg/10 ml.	Amount recovered mg.	% Recovery
0.40 mg. = 0.188 mg. Tetracycline HCl = 0.190 mg. Tetracycline HCl (as 99.0%)	0.125	0.315	0.315 ± 0.002	0.125 ± 0.002	100.0 ± 1.9
	0.250	0.440	0.442 ± 0.004	0.252 ± 0.004	100.8 ± 1.6
	0.375	0.565	0.566 ± 0.009	0.377 ± 0.009	100.5 ± 2.5
	0.500	0.690	0.694 ± 0.008	0.505 ± 0.008	100.9 ± 1.6
	0.750	0.940	0.943 ± 0.019	0.753 ± 0.019	100.4 ± 2.6

Average = 100.6

Individual experimental results are presented in Appendix II, Table III.

Each result is an average of five determinations ± Standard Deviation of the Mean.



4. Achromycin Capsule (Lederle Laboratories, Canada)

Each capsule is stated to contain 250 mg. tetracycline HCl.

The weights of the contents of ten capsules were taken separately and the mean was determined. The fine powder of the capsule was not completely soluble in water and the addition of 3M HCl in this instance to make an acidic pH of about 3.50 was found to cause a slight increase in diffusion current. This suggested that the solubility of tetracycline HCl in water, in which it is freely soluble, was decreased slightly by the presence of some other material. This situation was obtained only in the case of Achromycin capsule and was not obtained in any other tetracycline or other antibiotic preparations. The results are shown in Tables X and XI.

5. Achromycin Syrup (Lederle Laboratories, Canada)

The cherry flavoured syrup is buffered with citric acid and sodium citrate and each ml. is stated to contain 25 mg. tetracycline HCl and 0.12% methyl paraben and 0.03% propyl paraben.

It is a heavy cherry colored syrup. It was diluted with water and with the addition of a few drops of 3M HCl a lighter suspension was obtained which it was felt ensured complete solution of the antibiotic.

The presence of citric acid and sodium citrate did not have any interfering effect on the waves of the pure tetracycline HCl. The analysis was carried out as usual using the diluted solution. The results are shown in Tables XII and XIII.

Table X

Results on the Polarographic Determination of the Concentration of Tetracycline Hydrochloride in Achromycin Capsule (Lederle - 250 mg./capsule).

Amount of the preparation mg./10 ml.	Tetracycline HCl found mg./10 ml.	Tetracycline HCl/capsule mg.	% Stated Concentration
0.25	0.163 ± 0.004	251.5 ± 5.9	100.6 ± 2.4
0.50	0.328 ± 0.008	253.5 ± 6.3	101.4 ± 2.5
0.75	0.497 ± 0.010	255.9 ± 5.2	102.3 ± 2.1
1.00	0.655 ± 0.015	252.8 ± 5.8	101.1 ± 2.3
1.50	0.981 ± 0.022	252.5 ± 5.5	101.0 ± 2.2

Average = 101.3

Individual experimental results are presented in Appendix I, Table IV.

Each result is an average of five determinations ± Standard Deviation of the Mean.

Table XI

Results on the Polarographic Determination of the Recovery of Added Tetracycline Hydrochloride from Achromycin Capsule.

Amount of the preparation mg./10 ml.	Tetracycline HCl (99.0%)				
	Amount added mg.	Total present mg./10 ml.	Total found mg./10 ml.	Amount recovered mg.	% Recovery
0.40 mg. ± 0.262 mg. Tetracycline HCl ± 0.265 mg. Tetracycline HCl (as 99.0%)	0.125	0.390	0.389 ± 0.002	0.124 ± 0.002	99.0 ± 1.5
	0.250	0.515	0.513 ± 0.006	0.248 ± 0.006	99.4 ± 2.4
	0.375	0.640	0.640 ± 0.006	0.375 ± 0.006	100.0 ± 1.6
	0.500	0.765	0.759 ± 0.012	0.494 ± 0.012	98.9 ± 2.3
	0.750	1.015	1.010 ± 0.014	0.746 ± 0.014	99.4 ± 1.9

Average = 99.3

Individual experimental results are presented in Appendix II, Table IV.

Each result is an average of five determinations ± Standard Deviation of the Mean.



Table XII

Results on the Polarographic Determination of the Concentration of Tetracycline Hydrochloride in Achromycin Syrup (Lederle - 25 mg./ml.)

Amount of the preparation ml./10 ml.	Tetracycline HCl found mg./10 ml.	Tetracycline HCl/ml mg.	% Stated Concentration
0.005	0.124 ± 0.003	24.8 ± 0.6	99.0 ± 2.5
0.010	0.249 ± 0.006	24.9 ± 0.6	99.6 ± 2.4
0.020	0.498 ± 0.008	24.9 ± 0.4	99.6 ± 1.7
0.030	0.752 ± 0.014	25.0 ± 0.4	100.2 ± 1.8
0.040	0.995 ± 0.015	24.9 ± 0.4	99.5 ± 1.5

Average = 99.6

Individual experimental results are presented in Appendix I, Table V.

Each result is an average of five determinations ± Standard Deviation of the Mean.

Table XIII

Results on the Polarographic Determination of the Recovery of Added Tetracycline Hydrochloride from Achromycin Syrup.

Amount of the preparation ml./10 ml.	Tetracycline HCl (99.0%)				
	Amount added mg.	Total present mg./10 ml.	Total found mg./10 ml.	Amount recovered mg.	% Recovery
0.01 ml. = 0.249 mg. Tetracycline HCl = 0.251 mg. Tetracycline HCl (as 99.0%)	0.125	0.376	0.376 ± 0.004	0.124 ± 0.004	99.7 ± 2.8
	0.250	0.501	0.500 ± 0.004	0.249 ± 0.004	99.5 ± 1.5
	0.375	0.626	0.624 ± 0.006	0.372 ± 0.006	99.2 ± 1.6
	0.500	0.751	0.750 ± 0.009	0.498 ± 0.009	99.7 ± 1.7
	0.750	1.001	0.997 ± 0.014	0.745 ± 0.014	99.4 ± 1.9

Average = 99.5

Individual experimental results are presented in Appendix II, Table V.
Each result is an average of five determinations ± Standard Deviation of the Mean.

6. Achrocidin Tablet (Lederle Laboratories, Canada)

The stated formula for the tablet is

Tetracycline HCl	125 mg.
Phenacetin	120 mg.
Caffeine	30 mg.
Salicylamide	150 mg.
Chlorothen citrate	25 mg.

Of all these components, phenacetin alone showed a barely defined wave under the conditions used for the measurement of tetracycline HCl. The wave was apparent only when the concentration of the phenacetin was at 5.00 mg/10 ml or higher. At lower concentration, it manifested itself only as a small increase in the residual current. Some of the other components caused a very minimal increase in the residual current. Because of these complications, a blank containing all these components was run to obtain a true measure of the tetracycline present. It is to be noted that with this preparation measurements must be confined to the first wave only since the peak of the second wave was not sharp due to a high rise of the blank at -1.55 v. The results are shown in Tables XIV and XV.

7. Achrocidin Compound Syrup (Lederle Laboratories, Canada)

Each ml of the syrup is stated to contain

Table XIV

Results on the Polarographic Determination of the Concentration of Tetracycline Hydrochloride in Achrocidin Tablet (Lederle - 125 mg./tablet)

Amount of the preparation mg/10 ml.	Tetracycline HCl found mg/10 ml.	Tetracycline HCl/tablet mg.	% Stated Concentration
1.25	0.201 ± 0.004	125.8 ± 2.4	100.7 ± 2.0
2.50	0.399 ± 0.008	125.0 ± 2.6	100.0 ± 2.1
3.75	0.599 ± 0.010	125.1 ± 2.0	100.1 ± 1.6
5.00	0.795 ± 0.014	124.6 ± 2.2	99.7 ± 1.8
6.25	0.999 ± 0.020	125.2 ± 2.5	100.2 ± 2.0

Average = 100.1

Individual experimental results are presented in Appendix I, Table VI.

Each result is an average of five determinations ± Standard Deviation of the Mean.

Table XV

Results on the Polarographic Determination of the Recovery of Added Tetracycline Hydrochloride from Achrocidin Tablet.

Amount of the preparation mg./10 ml.	Tetracycline HCl (99.0%)				
	Amount added mg.	Total present mg./10 ml.	Total found mg./10 ml.	Amount recovered mg.	% Recovery
1.50 mg. = 0.242 mg. Tetracycline HCl = 0.244 mg. Tetracycline HCl (as 99.0%)	0.125	0.369	0.370 ± 0.002	0.126 ± 0.002	100.5 ± 2.0
	0.250	0.494	0.496 ± 0.006	0.252 ± 0.006	100.8 ± 2.4
	0.375	0.619	0.627 ± 0.006	0.377 ± 0.006	100.6 ± 1.6
	0.500	0.744	0.744 ± 0.011	0.499 ± 0.011	99.8 ± 2.2
	0.750	0.994	0.996 ± 0.017	0.752 ± 0.017	100.2 ± 2.2

Average = 100.4

Individual experimental results are presented in Appendix II, Table VI.

Each result is an average of five determinations ± Standard Deviation of the Mean.

Tetracycline HCl	25 mg.
Phenacetin	24 mg.
Salicylamide	30 mg.
Ascorbic acid	5 mg.
Pyrilamine maleate	3 mg.
Methyl paraben	0.8 mg.
Propyl paraben	0.2 mg.

This is a cherry flavoured heavy syrup containing some whitish insoluble materials. Under the standard conditions previously established, each of the different components was polarographed to ascertain any possible interfering effect which they might contribute. Phenacetin again in high concentration showed a small wave as discussed before. Ascorbic acid was found to have a well-defined wave but at a much less negative potential ($E\ 1/2 = -0.15$ v) than that of tetracycline. Thus the effect of ascorbic acid could be eliminated readily from the polarographic wave of tetracycline. None of the other components were found to produce any wave under the conditions of the test, except for a slight increase in the residual current. The blank containing all these components showed, however, a sharp rise which interfered with the determination of the second peak for tetracycline HCl thus limiting the measurement to that of the first wave. Again the first wave is an effective measurement of the concentration of tetracycline HCl.

The vial containing the preparation was shaken thoroughly and a definite volume of the syrup was diluted with water. A few drops of 3M HCl was added to make certain that complete solution of the tetracycline was obtained. The results are shown in Tables XVI and XVII.

8. Tetrex Capsule (Bristol Laboratories of Canada Ltd.)

Each capsule is stated to contain tetracycline phosphate complex equivalent to 100 mg. tetracycline HCl.

The analysis of pure tetracycline HCl was carried out in borate buffer. To note whether there was any effect due to the presence of phosphate on the waves obtained with borate buffer, disodium hydrogen phosphate was added in varying amounts to the solution of tetracycline HCl analysed. It was found that unless the amount was greatly in excess of the amount present in the preparation there was no effect on the waves of tetracycline HCl. Sodium acid phosphate had the same effect. On the other hand, if analysis of tetracycline HCl was carried out in phosphate buffer at the same pH the waves formed were slightly different from those obtained with borate buffer and a maximum appeared at the second wave. Addition of borate buffer to the solution in phosphate buffer resulted in the waves becoming progressively more like those obtained with borate buffer.

The proportion of phosphate present in the capsule did not have any effect on the waves of tetracycline HCl obtained with borate buffer. To remove any possible difference due to the presence of phosphate, disodium hydrogen phosphate in the possible equimolar

Table XVI

Results on the Polarographic Determination of the Concentration of Tetracycline Hydrochloride in Achrocidin Compound Syrup (Lederle - 25 mg./ml.)

Amount of the preparation ml/10 ml.	Tetracycline HCl found mg/10 ml.	Tetracycline HCl/ml mg.	% Stated Concentration
0.005	0.131 ± 0.004	26.3 ± 0.7	105.0 ± 3.0
0.010	0.262 ± 0.006	26.2 ± 0.6	104.7 ± 2.5
0.020	0.522 ± 0.009	26.1 ± 0.4	104.4 ± 1.8
0.030	0.787 ± 0.012	26.2 ± 0.4	104.9 ± 1.5
0.040	1.046 ± 0.019	26.2 ± 0.5	104.6 ± 1.9

Average = 104.7

Individual experimental results are presented in Appendix I, Table VII.

Each result is an average of five determinations ± Standard Deviation of the Mean.

Table XVII

Results on the Polarographic Determination of the Recovery of Added Tetracycline Hydrochloride from Achrocidin Compound Syrup.

Amount of the preparation ml./10 ml.	Tetracycline HCl (99.0%)				
	Amount added mg.	Total present mg./10 ml.	Total found mg./10 ml.	Amount recovered mg.	% Recovery
0.01 ml. = 0.262 mg. Tetracycline HCl = 0.264 mg. Tetracycline HCl (as 99.0%)	0.125	0.389	0.390 ± 0.003	0.126 ± 0.003	100.5 ± 2.5
	0.250	0.514	0.513 ± 0.006	0.249 ± 0.006	99.5 ± 2.4
	0.375	0.639	0.639 ± 0.007	0.374 ± 0.007	99.8 ± 1.9
	0.500	0.764	0.765 ± 0.012	0.500 ± 0.012	100.0 ± 2.3
	0.750	1.014	1.011 ± 0.017	0.746 ± 0.017	99.5 ± 2.2

Average = 99.9

Individual experimental results are presented in Appendix II, Table VII.

Each result is an average of five determinations ± Standard Deviation of the Mean.

proportion in which it is present in the capsule was added to the standard solution of the pure antibiotic. To a known amount of the powder isopropanol was added until the powder dissolved and water was added to make up the volume. The same volume of the isopropanol solvent was added to the standard solution of tetracycline HCl. The proportion of isopropanol in the final solution analysed was less than 0.1% and did not make the media non-aqueous. The results are shown in Tables XVIII and XIX.

9. Tetrex I.M. (Bristol Laboratories of Canada Limited)

Each vial is stated to contain

Tetracycline phosphate complex equivalent to	250 mg. tetracycline HCl
Xylocaine HCl	40 mg.
Magnesium chloride	46 mg.
Ascorbic acid	300 mg.

Xylocaine in a concentration of 5.00 mg/10 ml. gave a very small wave at -1.15v in borate buffer at pH 7.75. But the proportion in which it is present in the preparation had very little effect since the measurements were generally limited to a maximum concentration of 1.00 mg tetracycline HCl/10 ml. Magnesium chloride was found to have a marked effect on the two waves of tetracycline HCl. The first wave was slightly decreased and the second wave was increased due probably to some complex formation between the magnesium ion and tetracycline HCl. Addition of ethylenediaminetetraacetic acid disodium salt (EDTA) gradually increased the first wave and decreased the second

Table XVIII

Results on the Polarographic Determination of the Concentration of Tetracycline Hydrochloride in Tetrex Capsule (Bristol - 100 mg/capsule)

Amount of the preparation mg./10 ml.	Tetracycline HCl found mg./10 ml.	Tetracycline HCl/capsule mg.	% Stated Concentration
0.50	0.214 ± 0.004	106.4 ± 1.8	106.4 ± 1.8
0.75	0.321 ± 0.006	106.1 ± 2.0	106. 1 ± 2.0
1.00	0.429 ± 0.009	106.4 ± 2.3	106.4 ± 2.3
1.50	0.634 ± 0.015	104.9 ± 2.5	104.9 ± 2.5
2.00	0.856 ± 0.014	106.2 ± 1.7	106.2 ± 1.7

Average = 106.0

Individual experimental results are presented in Appendix I, Table VIII.

Each result is an average of five determinations ± Standard Deviation of the Mean.

Table XIX

Results on the Polarographic Determination of the Recovery of Added Tetracycline Hydrochloride from Tetrex Capsule.

Amount of the preparation mg./10 ml.	Tetracycline HCl (99.0%)				
	Amount added mg.	Total present mg./10 ml.	Total found mg./10 ml.	Amount recovered mg.	% Recovery
0.50 mg. ≡ 0.214 mg. Tetracycline HCl = 0.216 mg. Tetracycline HCl (as 99.0%)	0.125	0.341	0.340 ± 0.004	0.124 ± 0.004	99.4 ± 3.1
	0.250	0.466	0.465 ± 0.005	0.249 ± 0.005	99.7 ± 2.0
	0.375	0.591	0.588 ± 0.006	0.373 ± 0.006	99.4 ± 1.6
	0.500	0.716	0.714 ± 0.011	0.498 ± 0.011	99.6 ± 2.2
	0.750	0.966	0.965 ± 0.010	0.749 ± 0.010	99.9 ± 1.3

Average = 99.6

Individual experimental results are presented in Appendix II, Table VIII.

Each result is an average of five determinations ± Standard Deviation of the Mean.

until at equimolar concentration of magnesium chloride and EDTA the waves became normal for pure tetracycline HCl and there was no further change with further increase in concentration of EDTA. EDTA itself did not have any effect on the waves of tetracycline HCl nor did its chelate with magnesium.

Ascorbic acid had a distinct wave which was complete at -.15 v, after which there was a definite limiting current which persisted up to the waves of tetracycline HCl.

A blank containing all these components was run to eliminate any minor error in the residual current of the waves of tetracycline HCl. The material of the vial was weighed and a known amount was taken in water and a few drops of 3M HCl was added to ensure complete solution of the tetracycline. Using three times the equivalent concentration of EDTA than the highest concentration of magnesium chloride present in the pharmaceutical preparation used, analysis was carried out in the usual way. The results appear in Tables XX and XXI.

10. Azotrex (Bristol Laboratories of Canada Limited)

Each capsule is stated to contain

Tetracycline phosphate complex equivalent to	125 mg. tetracycline HCl
Sulfamethizole	250 mg.
Phenazopyridine HCl	50 mg.

The powder of the capsule was not completely soluble even in the acidic pH range. A few drops of 3M HCl was added to the

Table XX

Results on the Polarographic Determination of the Concentration of Tetracycline Hydrochloride in Tetrex I.M. (Bristol - 250 ng./vial)

Amount of the preparation mg./10 ml.	Tetracycline HCl found mg./10 ml.	Tetracycline HCl/vial mg.	% Stated Concentration
0.438	0.126 ± 0.004	259.1 ± 7.4	103.6 ± 3.0
0.875	0.252 ± 0.004	258.2 ± 3.8	103.3 ± 1.5
1.750	0.502 ± 0.011	257.6 ± 5.7	103.0 ± 2.3
2.625	0.751 ± 0.014	256.9 ± 4.9	102.8 ± 1.9
3.50	1.008 ± 0.02	258.4 ± 5.0	103.4 ± 2.0

Average = 103.2

Individual experimental results are presented in Appendix I, Table IX.

Each result is an average of five determinations ± Standard Deviation of the Mean.

Table XXI

Results on the Polarographic Determination of the Recovery of Added Tetracycline Hydrochloride from Tetrex I.M.

Amount of the preparation mg./10 ml.	Tetracycline HCl (99.0%)				
	Amount added mg.	Total present mg./10 ml.	Total found mg./10 ml.	Amount recovered mg.	% Recovery
1.00 mg. = 0.288 mg. Tetracycline HCl = 0.290 mg. Tetracycline HCl (as 99.0%)	0.125	0.415	0.415 ± 0.004	0.125 ± 0.004	100.0 ± 3.2
	0.250	0.540	0.542 ± 0.006	0.251 ± 0.006	100.5 ± 2.4
	0.375	0.665	0.665 ± 0.006	0.375 ± 0.006	100.0 ± 1.6
	0.500	0.790	0.793 ± 0.008	0.503 ± 0.008	100.5 ± 1.7
	0.750	1.040	1.043 ± 0.015	0.753 ± 0.015	100.3 ± 2.0

Average = 100.2

Individual experimental results are presented in Appendix II, Table IX.

Each result is an average of five determinations ± Standard Deviation of the Mean.

suspension of the powder in water to ensure complete solution of the antibiotic. Insoluble materials settled to the bottom.

Sulfamethizole did not give any wave in the region of -1.00 to -1.50 v but concentration of 3.00 mg/10 ml. and higher showed a slight rise from -1.35 v. A blank containing the two known components of the preparation and disodium hydrogen phosphate for reasons already mentioned under Tetrex (page 63) was run to remove any possible interfering effect of them on the waves of tetracycline HCl, and the measurement was limited to the first wave. The results appear in Tables XXII and XXIII.

11. Albamycin T Tablet (The Upjohn Company, Canada)

Each yellow coloured tablet is stated to contain

Novobiocin (as Albamycin calcium)	125 mg.
Tetracycline HCl	125 mg.

Novobiocin did not possess any polarographic reduction wave at any pH studied. It was therefore possible to determine only the amount of tetracycline HCl present in Albamycin T tablet.

The powder obtained by grinding the tablet in a mortar was not completely soluble in either the acid or alkaline pH range. The suspension in water was made acidic by adding a few drops of 3M HCl to ensure complete solution of the tetracycline HCl. The second wave was not very suitable for quantitative measurements, and the measurement

Table XXII

Results on the Polarographic Determination of the Concentration of Tetracycline Hydrochloride in Azotrex (Bristol - 125 mg./capsule)

Amount of the preparation mg./10 ml.	Tetracycline HCl found mg./10 ml.	Tetracycline HCl/capsule mg.	% Stated Concentration
0.50	0.125 ± 0.004	129.8 ± 3.8	103.8 ± 3.1
1.00	0.252 ± 0.006	130.5 ± 3.2	104.4 ± 2.5
1.50	0.376 ± 0.006	129.9 ± 2.2	103.9 ± 1.8
2.00	0.501 ± 0.010	129.9 ± 2.5	103.9 ± 2.0
3.00	0.750 ± 0.012	129.6 ± 2.0	103.7 ± 1.6

Average = 104.0

Individual experimental results are presented in Appendix I, Table X.

Each result is an average of five determinations ± Standard Deviation of the Mean.

Table XXIII

Results on the Polarographic Determination of the Recovery of Added Tetracycline Hydrochloride from Azotrex.

Amount of the preparation mg./10 ml.	Tetracycline HCl (99.0%)				
	Amount added mg.	Total present mg./10 ml.	Total found mg./10 ml.	Amount recovered mg.	% Recovery
1.0 mg. = 0.251 mg. Tetracycline HCl = 0.253 mg. Tetracycline HCl (as 99.0%)	0.125	0.378	0.379 ± 0.003	0.126 ± 0.003	100.6 ± 2.6
	0.250	0.503	0.500 ± 0.006	0.247 ± 0.006	98.8 ± 2.4
	0.375	0.628	0.626 ± 0.011	0.374 ± 0.011	99.6 ± 3.0
	0.500	0.753	0.750 ± 0.012	0.497 ± 0.012	99.4 ± 2.3
	0.750	1.003	1.002 ± 0.014	0.748 ± 0.014	99.8 ± 1.9

Average = 99.6 ± 0.6

Individual experimental results are presented in Appendix II, Table X.

Each result is an average of five determinations ± Standard Deviation of the Mean.

was limited to the first wave only. The results appear in Tables XXIV and XXV.

12. Albamycin T Granules (The Upjohn Company, Canada)

Each vial is stated to contain

Tetracycline base equivalent to 62.50 mg. tetracycline HCl

Albamycin base 62.50 mg.

The contents of a few vials were weighed separately and the mean weight was determined. The flavoured granules were powdered finely in a mortar. The procedure was the same as for Albamycin T tablet and quantitative measurements were limited to the first wave since the second wave was not very suitable. The results appear in Tables XXVI and XXVII.

(ii) Chlorotetracycline Hydrochloride

In 1952, Doskocil and Vondracek (132) were the earliest workers to use polarography for the analysis of Cl-tetracycline. Doskocil (133) two years later reported some modifications of the conditions of their polarographic method for simultaneous determination of Cl-tetracycline and oxytetracycline. The polarographic behaviour of Cl-tetracycline was also studied by Krestynova-Tellupilova, Macak and Santavy (134).

The use of oscillography for the identification of tetracycline, Cl-tetracycline and oxytetracycline has already been mentioned (131).

Table XXIV

Results on the Polarographic Determination of the Concentration of Tetracycline Hydrochloride in Albamycin T Tablet (Upjohn - 125 mg./tablet)

Amount of the preparation mg./10 ml.	Tetracycline HCl found mg./10 ml.	Tetracycline HCl/tablet mg.	% Stated Concentration
0.50	0.127 ± 0.003	129.5 ± 3.0	103.6 ± 2.4
1.00	0.256 ± 0.006	130.2 ± 3.0	104.1 ± 2.4
2.00	0.508 ± 0.011	129.0 ± 2.9	103.2 ± 2.3
3.00	0.759 ± 0.017	128.6 ± 2.9	102.9 ± 2.3
4.00	1.014 ± 0.021	128.8 ± 2.7	103.1 ± 2.1

Average = 103.4

Individual experimental results are presented in Appendix I, Table XI.

Each result is an average of five determinations ± Standard Deviation of the Mean.

Table XXV

Results on the Polarographic Determination of the Recovery of Added Tetracycline Hydrochloride from Albamycin T Tablet.

Amount of the preparation mg./10 ml.	Tetracycline HCl (99.0%)				
	Amount added mg.	Total present mg./10 ml.	Total found mg./10 ml.	Amount recovered mg.	% Recovery
1.0 mg. = 0.254 mg. Tetracycline HCl = 0.257 mg. Tetracycline HCl (as 99.0%)	0.125	0.382	0.381 ± 0.003	0.124 ± 0.003	99.5 ± 2.4
	0.250	0.507	0.508 ± 0.006	0.251 ± 0.006	100.4 ± 2.4
	0.375	0.632	0.630 ± 0.008	0.373 ± 0.008	99.4 ± 2.2
	0.500	0.757	0.753 ± 0.009	0.496 ± 0.009	99.3 ± 1.8
	0.750	1.007	1.002 ± 0.012	0.745 ± 0.012	99.3 ± 1.6

Average = 99.6

Individual experimental results are presented in Appendix II, Table XI.

Each result is an average of five determinations ± Standard Deviation of the Mean.

Table XXVI

Results on the Polarographic Determination of the Concentration of Tetracycline Hydrochloride in Albamycin T Granules (Upjohn - 62.5 mg./vial)

Amount of the preparation mg./10 ml.	Tetracycline HCl found mg./10 ml.	Tetracycline HCl/vial mg.	% Stated Concentration
5.00	0.256 ± 0.006	65.8 ± 1.6	105.2 ± 2.6
7.50	0.383 ± 0.008	65.6 ± 1.4	105.0 ± 2.3
10.00	0.511 ± 0.010	65.6 ± 1.2	104.9 ± 2.0
15.00	0.770 ± 0.015	65.9 ± 1.3	105.4 ± 2.1
20.00	1.026 ± 0.015	65.8 ± 1.0	105.4 ± 1.6

Average = 105.2

Individual experimental results are presented in Appendix I, Table XII.

Each result is an average of five determinations ± Standard Deviation of the Mean.

Table XXVII

Results on the Polarographic Determination of the Recovery of Added Tetracycline Hydrochloride from Albamycin T Granules.

Amount of the preparation mg./10 ml.	Tetracycline HCl (99.0%)				
	Amount added mg.	Total present mg./10 ml.	Total found mg./10 ml.	Amount recovered mg.	% Recovery
4.0 mg. ≡ 0.205 mg. Tetracycline HC l ≡ 0.207 mg. Tetracycline HCl (as 99.0%)	0.125	0.332	0.333 ± 0.003	0.126 ± 0.003	101.1 ± 2.2
	0.250	0.457	0.458 ± 0.006	0.251 ± 0.006	100.3 ± 2.3
	0.375	0.582	0.583 ± 0.010	0.376 ± 0.010	100.2 ± 2.6
	0.500	0.707	0.709 ± 0.008	0.502 ± 0.008	100.5 ± 1.7
	0.750	0.957	0.958 ± 0.013	0.751 ± 0.013	100.1 ± 1.7

Average = 100.5

Individual experimental results are presented in Appendix II, Table XII.

Each result is an average of five determinations ± Standard Deviation of the Mean.

Cl-tetracycline gave more or less similar waves to those of tetracycline and at similar potentials and the pH range of 6 to 9 was best suited for quantitative analysis. The resolution between the two waves in borate buffer, however, was less defined and for accurate measurements diffusion currents had to be measured over the combined first and second waves. In phosphate buffer, there appeared two well resolved waves in the pH range of 7.50 to 8.20 and the pH of 7.75 ± 0.05 was found most suitable for measuring diffusion currents. The half-wave potentials were -1.165vfor the first wave and -1.42 v for the second wave. Results on the polarographic measurements of Cl-tetracycline are shown in Table XXVIII and Figure 6.

(ii)a. Pharmaceutical Preparations

1. Aureomycin Capsule (Lederle Laboratories, Canada)

Each yellow colored sealed capsule is stated to contain 250 mg. tetracycline HCl B.P.

The contents of ten capsules were weighed separately and the mean weight was determined. The powder was not completely soluble in water. Either the suspension or supernatant liquid could be used for quantitative analysis under identical conditions of pH and buffer as for the pure antibiotic. The results appear in Tables XXIX and XXX.

Table XXVIII

Data on the Diffusion Current of the First Wave of Chlorotetracyline HCl in Phosphate Buffer at pH 7.75 ± .05. $m^{2/3}t^{1/6}$=1.68 $mg.^{2/3}Sec.^{-1/2}$

Concentration mg/10 ml.	Diffusion Current 100 units = 3 Ua.	Average ± S.D.M.
0.050	2.17 2.00 2.00 2.00 2.00	2.0 ± 0.1
0.125	4.00 4.00 4.25 4.00 4.00	4.0 ± 0.1
0.250	7.50 7.50 7.50 7.25 7.50	7.4 ± 0.1
0.375	10.50 10.75 10.75 11.00 10.75	10.8 ± 0.2
0.500	14.00 13.75 14.50 14.00 13.75	14.0 ± 0.3
0.750	21.50 21.00 20.50 21.25 20.75	21.0 ± 0.4
1.000	27.25 26.50 28.00 27.25 27.50	27.3 ± 0.5
1.250	33.25 34.50 34.00 34.75 33.75	34.0 ± 0.6

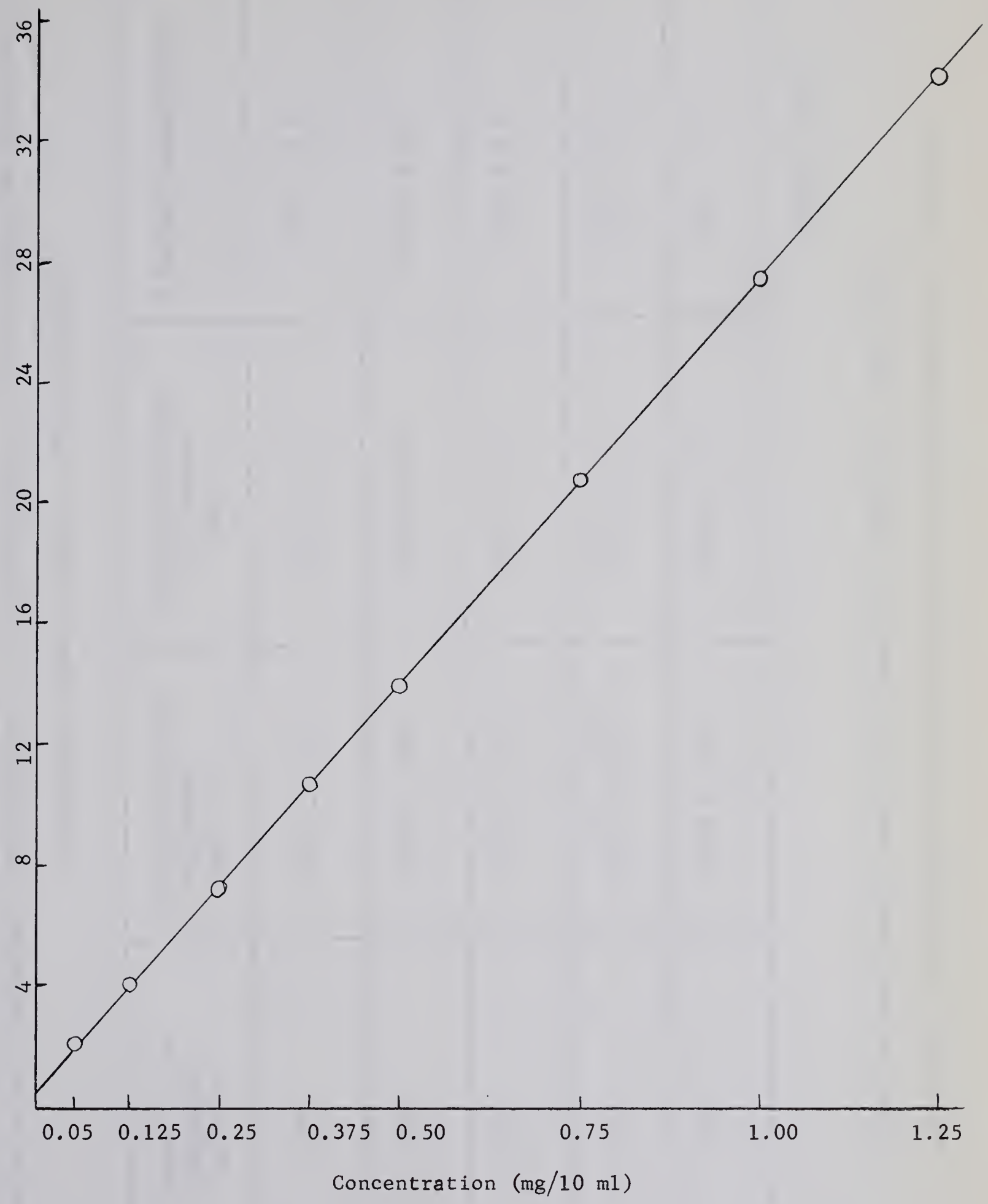


Figure 6. The graph of Cl-tetracycline Hydrochloride standard.

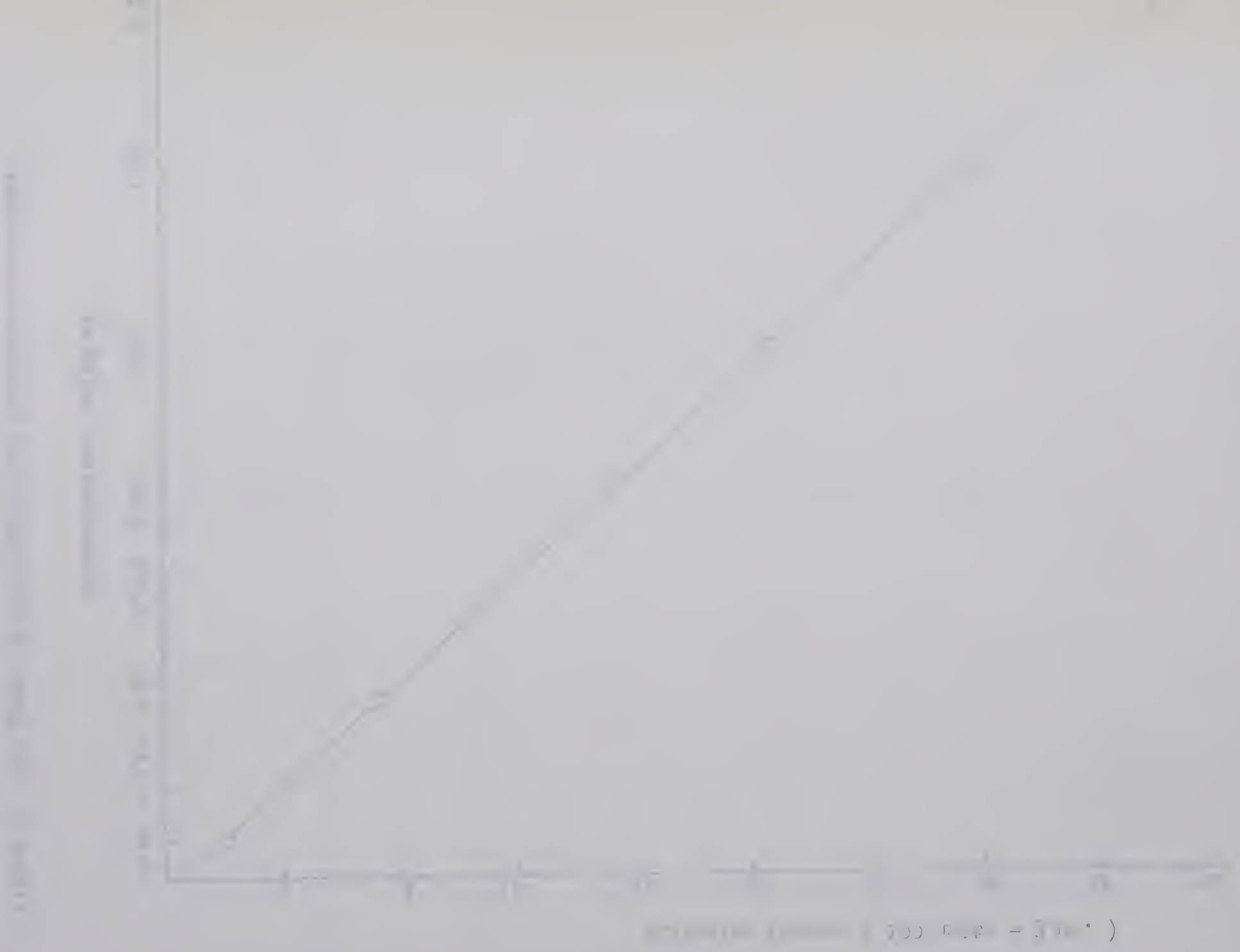


Table XXIX

Results on the Polarographic Determination of the Concentration of Chlorotetracycline Hydrochloride in Aureomycin Capsule (Lederle - 250 mg./capsule)

Amount of the preparation mg./10 ml.	Chlorotetracycline HCl found mg./10 ml.	Chlorotetracycline HCl/capsule mg.	% Stated Concentration
0.25	0.162 ± 0.003	256.7 ± 4.6	102.7 ± 1.8
0.50	0.326 ± 0.006	257.8 ± 4.6	103.1 ± 1.8
0.75	0.492 ± 0.013	258.9 ± 6.9	103.6 ± 2.8
1.00	0.656 ± 0.013	259.0 ± 5.1	103.6 ± 2.0
1.50	0.982 ± 0.014	258.6 ± 3.7	103.4 ± 1.5

Average = 103.3

Individual experimental results are presented in Appendix I, Table XIII.

Each result is an average of five determinations ± Standard Deviation of the Mean.

Table XXX

Results on the Polarographic Determination of the Recovery of Added Chlorotetracycline Hydrochloride from Aureomycin Capsule.

Amount of the preparation mg./10 ml.	Chlorotetracycline HCl (98.5%)				
	Amount added mg.	Total present mg/10 ml.	Total found mg./10 ml.	Amount recovered mg.	% Recovery
0.40 mg. = 0.262 mg. Cl-tetracycline HCl = 0.266 mg. Cl-tetracycline HCl (as 98.5%)	0.125	0.390	0.390 ± 0.002	0.124 ± 0.002	99.6 ± 1.9
	0.250	0.516	0.513 ± 0.007	0.248 ± 0.007	99.1 ± 3.0
	0.375	0.640	0.640 ± 0.009	0.374 ± 0.009	99.9 ± 2.3
	0.500	0.766	0.763 ± 0.012	0.498 ± 0.012	99.5 ± 2.4
	0.750	1.016	1.013 ± 0.015	0.748 ± 0.015	99.7 ± 2.0

Average = 99.6

Individual experimental results are presented in Appendix II, Table XIII.

Each result is an average of five determinations ± Standard Deviation of the Mean.

(iii) Demethyl Chlorotetracycline Hydrochloride (DMCT HCl)

There appears to be no published methods for the determination of this antibiotic since this is a relatively new member of the tetracyclines. DMCT HCl was found to give similar waves as those of tetracycline HCl but at slightly less negative potentials, and measurement could be carried out in the same pH range of 6 to 10 with either borate or phosphate buffer. However, to keep uniformity, borate buffer of pH 7.75 ± 0.05 was used for the analysis of this antibiotic.

Both the waves were well developed and diffusion current measurements could be made for either the first, the second or both waves together. The antibiotic was freely soluble in water. The E 1/2 of the first wave was -1.05 ± 0.01v and that for the second was -1.35 v. The results are shown in Table XXXI and Figure 7.

(iii)a. Pharmaceutical Preparations

1. Declomycin Capsule (Lederle Laboratories, Canada)

Each capsule is stated to contain 150 mg. DMCT HCl

The yellow powder was highly soluble in water and the rate of solution was even faster than that of the pure antibiotic. Using borate buffer of pH 7.75 ± 0.05 the analysis was carried out in the usual way. The waves for the pharmaceutical preparation were identical with those of the pure antibiotic, which indicated that there was no interference from any excipient or any other components. The results appear in Tables XXXII and XXXIII.

Table XXXI

Date on the Diffusion Current of the First Wave of Demethyl Chloro-tetracycline HCl (DMCT HCl) in Borate Buffer at pH 7.75 ± 0.05.

$m^{2/3} t^{1/6} = 1.69 \text{ mg.}^{2/3} \text{ Sec.}^{-1/2}$

Concentration mg/10 ml.	Diffusion Current 100 units = 3 µa.	Average ± S.D.M.
0.050	2.00 2.00 2.17 2.00 2.00	2.0 ± 0.1
0.125	4.25 4.50 4.25 4.25 4.25	4.3 ± 0.1
0.250	8.00 8.25 8.25 8.50 8.25	8.2 ± 0.2
0.375	11.75 12.00 11.50 11.50 11.75	11.7 ± 0.2
0.500	15.25 15.00 15.75 15.25 15.00	15.2 ± 0.3
0.750	22.50 23.00 22.00 22.75 22.25	22.5 ± 0.4
1.000	28.75 30.25 29.50 30.00 29.50	29.6 ± 0.6

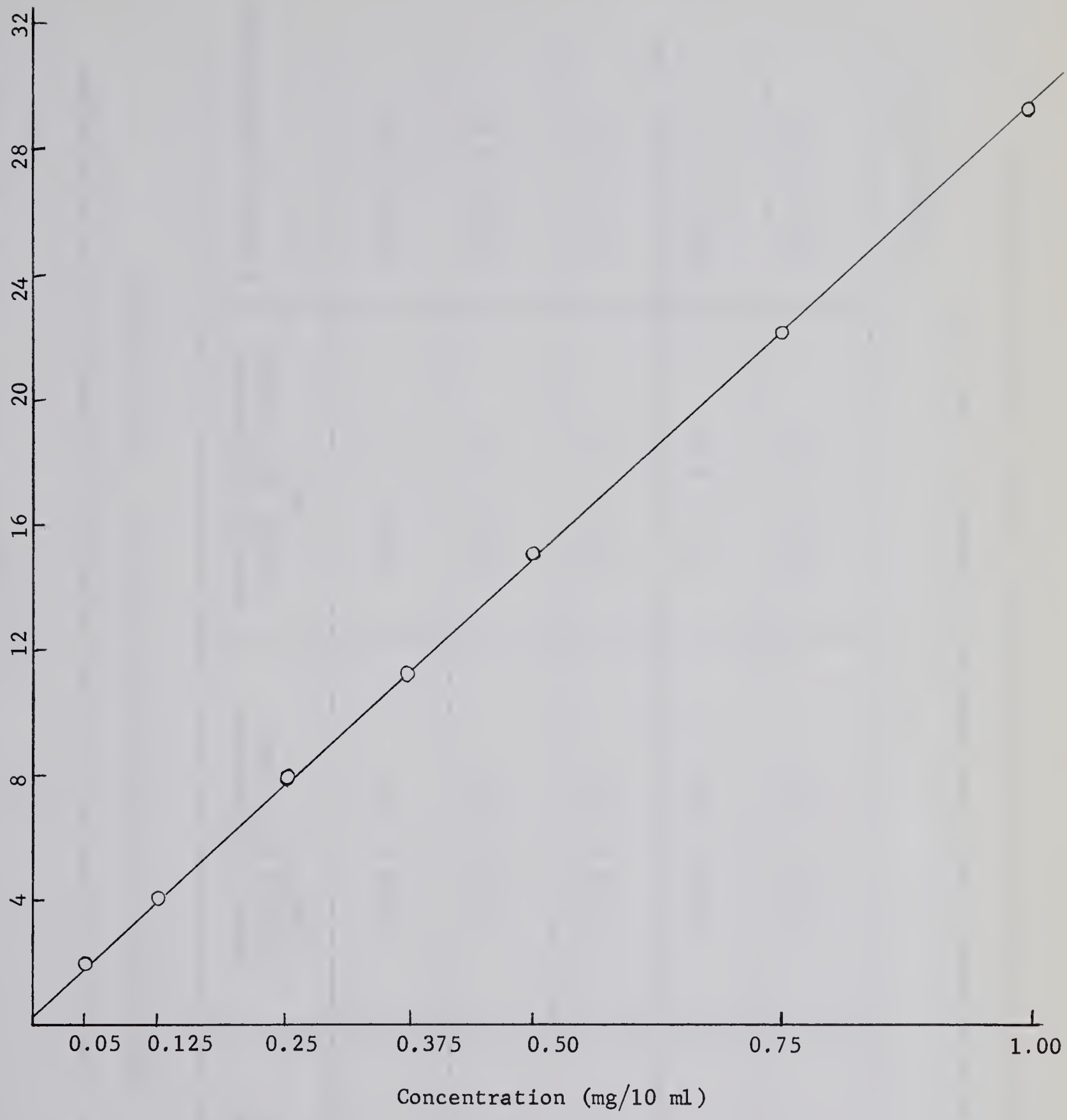


Figure 7. The graph of Demethyl Chlorotetracycline Hydrochloride standard.

Table XXXII

Results on the Polarographic Determination of the Concentration of Demethyl Chlorotetracycline Hydrochloride in Declomycin Capsule (Lederle - 150 mg./capsule)

Amount of the preparation mg./10 ml.	Demethyl Chlorotetracycline HCl found mg./10 ml.	Demethyl Chlorotetracycline HCl/capsule mg.	% Stated Concentration
0.50	0.229 ± 0.006	157.9 ± 4.3	105.3 ± 2.9
0.75	0.345 ± 0.006	158.7 ± 3.0	105.8 ± 2.0
1.00	0.455 ± 0.009	157.0 ± 3.1	104.7 ± 2.1
1.50	0.684 ± 0.013	157.4 ± 2.9	104.9 ± 2.0
2.00	0.912 ± 0.022	157.6 ± 3.9	105.0 ± 2.6

Average = 105.2

Individual experimental results are presented in Appendix I, Table XIV.

Each result is an average of five determinations ± Standard Deviation of the Mean.

Table XXXIII

Results on the Polarographic Determination of the Recovery of Added Demethyl Chlorotetracycline Hydrochloride from Declomycin Capsule.

Amount of the preparation mg./10 ml.	Demethyl Chlorotetracycline HCl (98.1%)				
	Amount added mg.	Total present mg./10 ml.	Total found mg./10 ml.	Amount recovered mg.	% Recovery
0.50 mg. = 0.228 mg. DMCT HCl = 0.233 mg. DMCT HCl (as 98.1%)	0.125	0.358	0.359 ± 0.004	0.126 ± 0.004	101.1 ± 2.9
	0.250	0.483	0.486 ± 0.004	0.253 ± 0.004	101.2 ± 1.8
	0.375	0.608	0.609 ± 0.007	0.376 ± 0.007	100.4 ± 1.8
	0.500	0.733	0.738 ± 0.009	0.505 ± 0.009	101.0 ± 1.9
	0.750	0.983	0.987 ± 0.015	0.755 ± 0.015	100.6 ± 2.0
				Average =	100.9

Individual experimental results are presented in Appendix II, Table XIV.

Each result is an average of five determinations ± Standard Deviation of the Mean.

2. Declomycin Syrup (cherry flavoured) (Lederle Laboratories, Canada)

The cherry-red syrup, which is quite acidic, is a heavy liquid and has a stated composition of

DMCT HCl	15.00 mg/ml
Methyl paraben	0.08%
Propyl paraben	0.02%

The bottle was shaken well and an aliquot of the syrup was diluted with water in a volumetric flask. A blank consisting of the two preservatives was used to remove any slight interfering effect they might have on the residual current. The results are shown in Tables XXXIV and XXXV.

3. Declostatin Capsule (Lederle Laboratories, Canada)

Each sealed capsule is stated to contain

DMCT HCl	150 mg.
Nystatin	250,000 units equivalent to 262.50 mg.

Nystatin like novobiocin did not have any polarographic reduction wave at any pH studied with potassium chloride, borate buffer or phosphate buffer as supporting electrolyte, and did not have any effect on the waves of DMCT HCl.

The powder was not completely soluble in water but was soluble in 15% ethanol. A standard solution was prepared in the same manner for analysis of this pharmaceutical preparation. The concentration of

Table XXXIV

Results on the Polarographic Determination of the Concentration of Demethyl Chlorotetracycline Hydrochloride in Declomycin Syrup (Lederle - 15 mg./ml.)

Amount of the preparation ml./10 ml.	Demethyl Chlorotetracycline HCl found mg./10 ml.	Demethyl Chlorotetracycline HCl/ml. mg.	% Stated Concentration
0.0125	0.196 ± 0.005	15.7 ± 0.4	104.7 ± 2.9
0.0250	0.394 ± 0.006	15.8 ± 0.3	105.1 ± 1.7
0.0375	0.593 ± 0.018	15.8 ± 0.5	105.5 ± 3.3
0.0500	0.786 ± 0.016	15.7 ± 0.3	104.9 ± 2.1
0.0625	0.984 ± 0.028	15.8 ± 0.4	105.0 ± 2.9

Average = 104.7

Individual experimental results are presented in Appendix I, Table XV.

Each result is an average of five determinations ± Standard Deviation of the Mean.

Table XXXV

Results on the Polarographic Determination of the Recovery of Added Demethyl Chlorotetracycline Hydrochloride from Declomycin Syrup.

Amount of the preparation ml./10 ml.	Demethyl Chlorotetracycline HCl (98.1%)				
	Amount added mg.	Total present mg./10 ml.	Total found mg./10 ml.	Amount recovered mg.	% Recovery
0.02 ml. = 0.314 mg. DMCT HCl = 0.320 mg. DMCT HCl (as 98.1%)	0.125	0.445	0.445 ± 0.004	0.125 ± 0.004	100.0 ± 3.4
	0.250	0.570	0.570 ± 0.006	0.250 ± 0.006	100.0 ± 2.3
	0.375	0.695	0.695 ± 0.007	0.375 ± 0.007	100.0 ± 1.9
	0.500	0.820	0.825 ± 0.011	0.505 ± 0.011	101.0 ± 2.2
	0.750	1.070	1.072 ± 0.013	0.752 ± 0.013	100.2 ± 1.8

Average = 100.2

Individual experimental results are presented in Appendix II, Table XV.

Each result is an average of five determinations ± Standard Deviation of the Mean.

alcohol in the final solution analysed was not more than 1%. The results are shown in Tables XXXVI and XXXVII.

(iv) Oxytetracycline Hydrochloride

The early polarographic work on oxytetracycline was done by Telupilova and Masinova (135), Doskocil (133) and Krestynova-Telupilova et al.(134). These workers studied the polarographic behaviour as well as conditions for quantitative measurement of this antibiotic.

Oxytetracycline behaved somewhat different than tetracycline, Cl-tetracycline and DMCT. At low pH range of 3 to 4 there were several poorly developed reduction waves. From pH 4 to 7.50 there appeared three waves at -.94, -1.21 and -1.42 v. Starting from pH 7.50 the first two waves combined into one. With increase in pH the resolution between the two waves became progressively better and a pH of 9.75 was found most suitable for measurement of the diffusion currents. Either phosphate or borate buffer could be used to control the pH within ± 0.05 of a unit. Borate buffer was, however, preferable because it resulted in a more defined curve.

Diffusion current measurements were more accurate for the first wave than the second since the region after the first wave was quite steep resulting in more error in the measurement of diffusion current at higher concentration. The E 1/2 values for the two waves were -1.37 ± 0.01 and -1.64 ± 0.01 v. The results are shown in Table XXXVIII

Table XXXVI

Results on the Polarographic Determination of the Concentration of Demethyl Chlorotetracycline Hydrochloride in Declostatin Capsule (Lederle - 150 mg./capsule)

Amount of the preparation mg/10 ml.	Demethyl Chlorotetracycline HCl found mg/10 ml.	Demethyl Chlorotetracycline HCl/capsule mg.	% Stated Concentration
0.25	0.144 ± 0.003	158.8 ± 3.6	105.9 ± 2.4
0.50	0.286 ± 0.006	158.0 ± 3.0	105.3 ± 2.0
1.00	0.571 ± 0.013	157.6 ± 3.5	105.5 ± 2.3
1.50	0.857 ± 0.015	157.7 ± 2.7	105.2 ± 1.8
2.00	1.137 ± 0.022	156.9 ± 3.1	104.6 ± 2.0

Average = 105.2

Individual experimental results are presented in Appendix I, Table XVI.

Each result is an average of five determinations ± Standard Deviation of the Mean.

Table XXXVII

Results on the Polarographic Determination of the Recovery of Added Demethyl Chlorotetracycline Hydrochloride from Declostatin Capsule.

Amount of the preparation mg./10 ml.	Demethyl Chlorotetracycline HCl (98.1%)				
	Amount added mg.	Total present mg./10 ml.	Total found mg./10 ml.	Amount recovered mg.	% Recovery
0.40 mg. = 0.229 mg. DMCT HCl = 0.233 mg. DMCT HCl (as 98.1%)	0.125	0.358	0.357 ± 0.002	0.124 ± 0.002	99.0 ± 1.4
	0.250	0.483	0.484 ± 0.007	0.251 ± 0.007	100.4 ± 2.7
	0.375	0.608	0.609 ± 0.007	0.376 ± 0.007	100.3 ± 1.8
	0.500	0.733	0.733 ± 0.010	0.500 ± 0.010	100.0 ± 2.0
	0.750	0.983	0.989 ± 0.011	0.753 ± 0.011	100.4 ± 1.4

Average = 100.00

Individual experimental results are presented in Appendix II, Table XVI.

Each result is an average of five determinations ± Standard Deviation of the Mean.



Table XXXVIII

Data on the Diffusion Currents of the First Wave of Oxytetracycline HCl in Borate Buffer at pH 9.75 ± 0.05. $m^{2/3} t^{1/6}$ = 1.65 mg. $^{2/3}$ Sec. $^{-1/2}$

Concentration mg/10 ml.	Diffusion Current 100 units = 3 µa.	Average ± S.D.M.
0.050	2.00 1.83 2.00 2.00 2.00	2.0 ± 0.0
0.125	3.75 3.75 3.75 3.75 4.00	3.8 ± 0.2
0.250	7.75 7.50 7.50 7.25 7.50	7.5 ± 0.2
0.500	14.00 14.50 14.75 14.50 14.50	14.4 ± 0.3
0.750	21.75 21.25 20.75 21.00 21.50	21.2 ± 0.4
1.00	29.00 29.75 29.25 28.50 28.75	29.0 ± 0.5
1.250	36.25 35.75 35.50 35.25 34.75	35.5 ± 0.6
1.500	42.00 42.75 41.50 42.75 41.75	42.0 ± 0.6

and Figure 8. The results of the microbiological comparison are shown in Table XXXIX and Figure 9.

Oxytetracycline Amphoteric

Oxytetracycline amphoteric gave the same kind of waves as oxytetracycline HCl with similar diffusion current for similar concentration under identical conditions. The results are shown in Table XL and Figure 10. The microbiological comparisons are presented in Table XLI and Figure 11.

(iv)a. Pharmaceutical Preparations

1. Terramycin Capsule (Pfizer Corporation, Canada)

Each yellow capsule is stated to contain

Oxytetracycline HCl equivalent to	250 mg. oxytetracycline
and glucosamine HCl	250 mg.

Glucosamine HCl did not show any wave in the region where oxytetracycline HCl gave its waves and did not have any effect on the waves of oxytetracycline HCl. The powder of the capsule was not completely soluble in water and addition of 3M acid or alkali did not improve the solubility. A few drops of 3 M HCl was added to ensure complete solution of oxytetracycline HCl. Either the suspension or supernatant liquid could be used for analysis and gave similar results. Measurement of the diffusion current of the first wave or both waves together could be used to obtain good quantitative results. The results are shown in Tables XLII and XLIII.

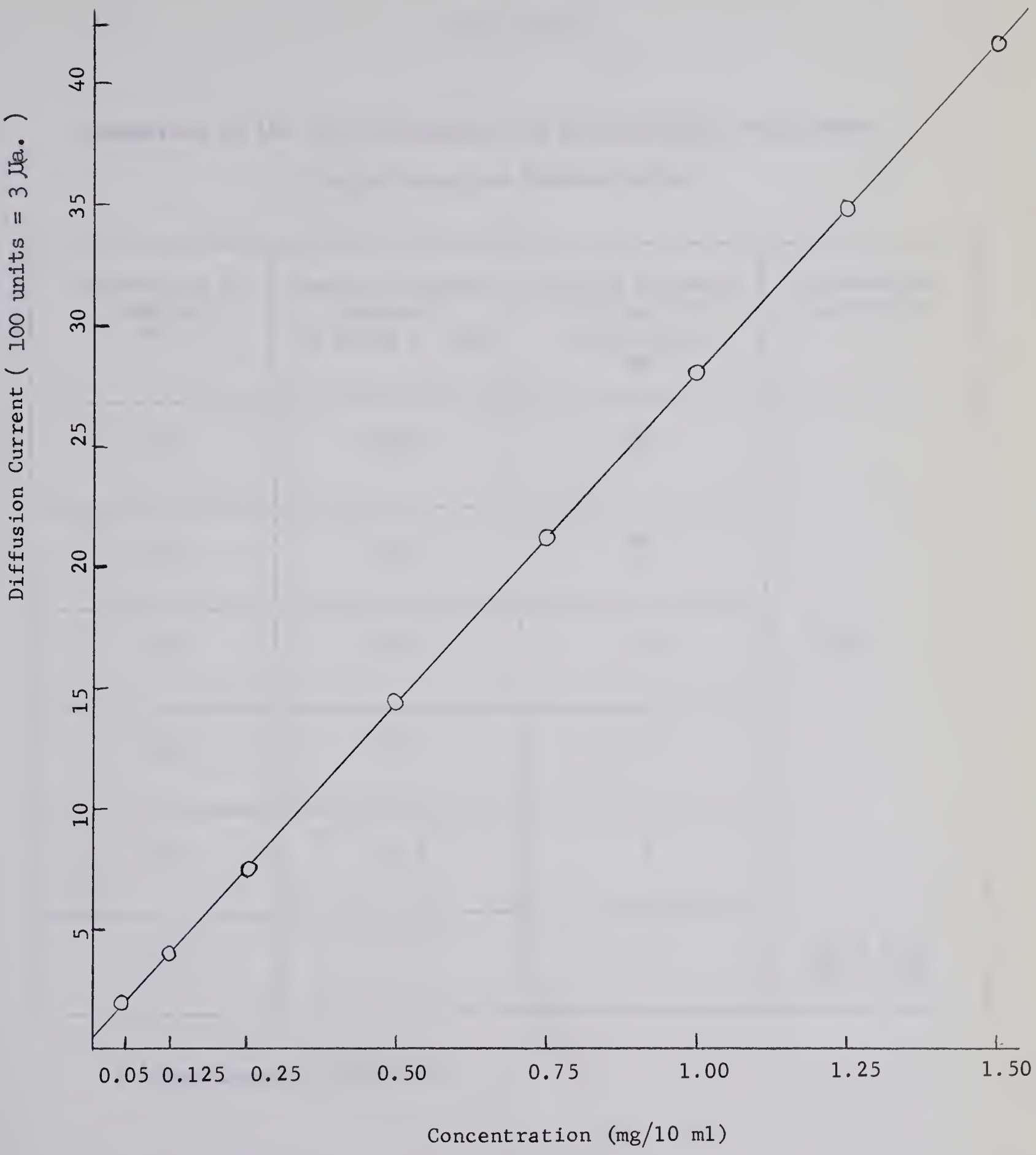


Figure 8. The graph of Oxytetracycline Hydrochloride standard.



Table XXXIX

Comparison of the Microbiological and Polarographic Measurement of Oxytetracycline Hydrochloride.

Temperature of Heating $0^{o}C$	Average Diffusion Current 100 Units = 3 μa.	Average Diameter of zone of inhibition mm.	Correlation Coefficient r
100	22.0	20	
110	22.0	20	
120	20.0	19	0.98*
130	11.4	14	
135	4.5	7	
140	0	0	95% = 0.95 99% = 0.99

* Significant at 95% level.

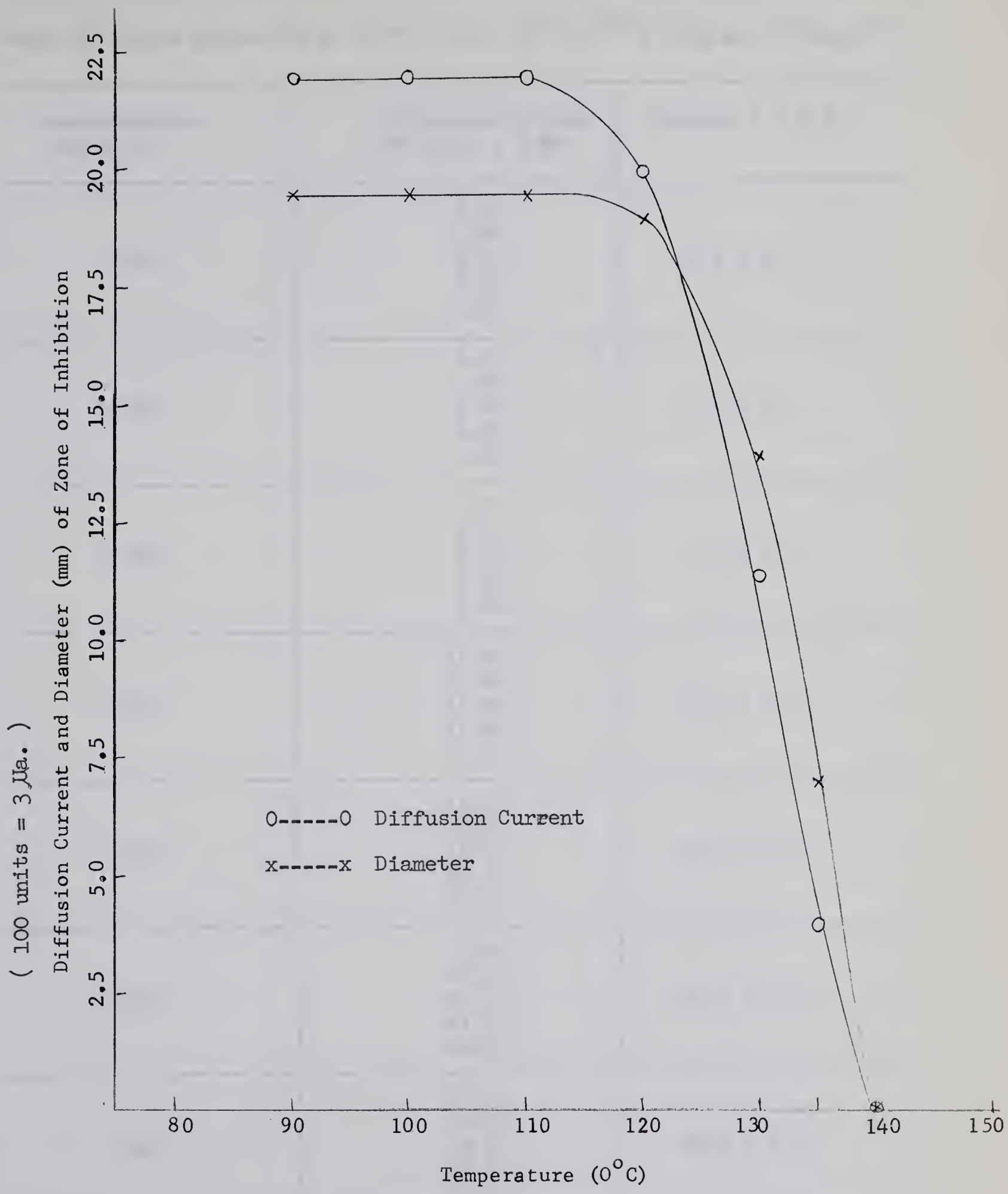


Figure 9. The comparison of microbiological and polarographic measurement of Oxytetracycline HCl during degradation.

Table XL

Data on the Diffusion Currents of the First Wave of Oxytetracycline Amphoteric in Borate Buffer at pH 9.75 ± 0.05. $m^{2/3} t^{1/6} = 1.65$ mg. $^{2/3}Sec.^{-1/2}$

Concentration mg/10 ml.	Diffusion Current 100 units = 3 µa.	Average ± S.D.M.
0.050	2.50 2.50 2.50 2.50 2.67	2.5 ± 0.1
0.125	4.25 4.00 4.25 4.25 4.25	4.2 ± 0.1
0.250	7.25 7.50 7.50 7.75 7.50	7.5 ± 0.2
0.375	11.25 11.00 11.25 11.25 11.75	11.3 ± 0.3
0.500	14.50 14.00 14.75 14.50 14.50	14.4 ± 0.3
0.750	22.00 22.50 21.50 21.75 22.25	22.0 ± 0.4
1.000	28.25 29.50 29.00 28.75 28.25	28.8 ± 0.5
1.250	35.75 36.50 36.00 35.50 35.00	35.8 ± 0.6

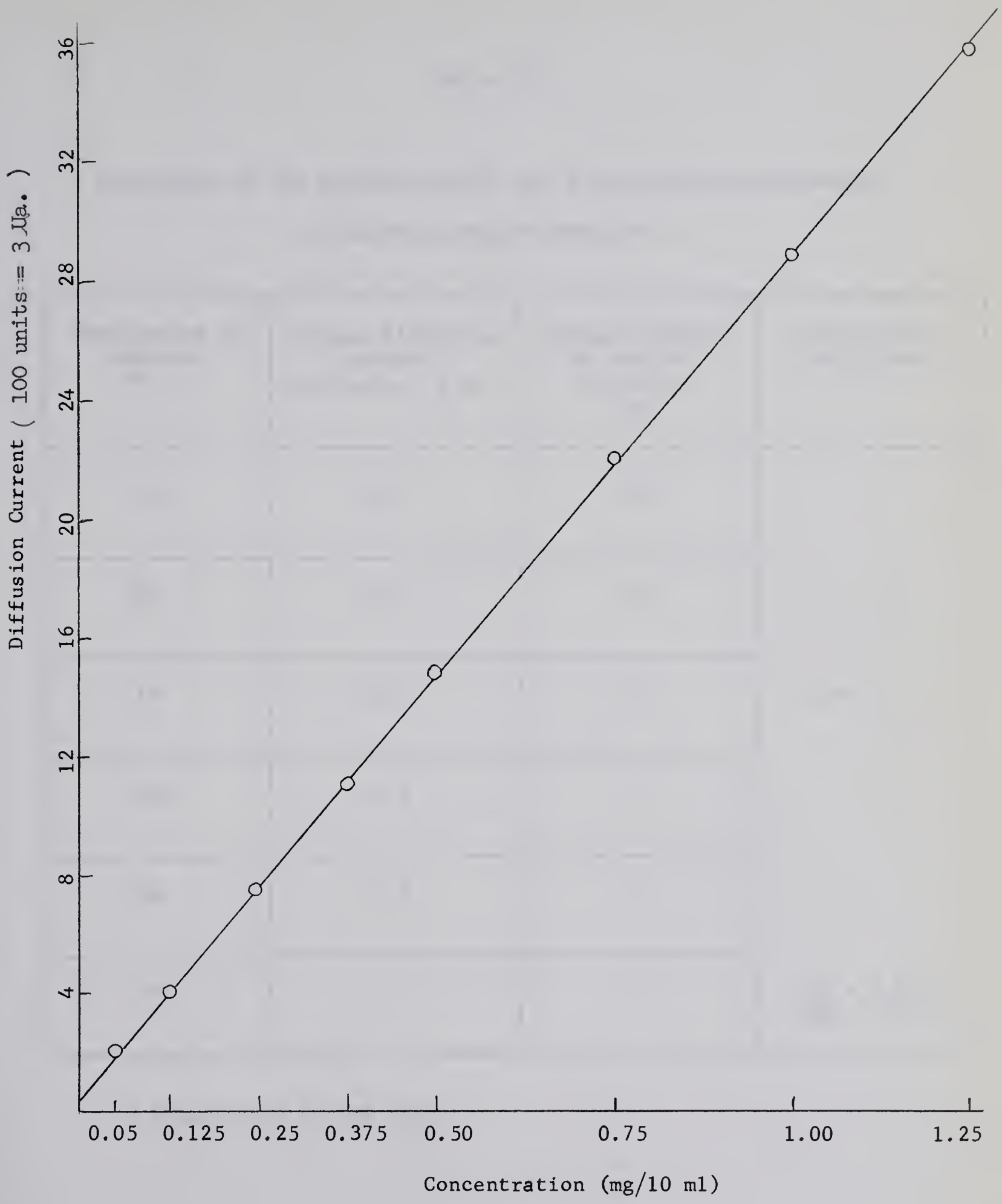


Figure 10. The graph of Oxytetracycline Amphoteric standard.

Table XLI

Comparison of the Microbiological and Polarographic Measurement of Oxytetracycline Amphoteric.

Temperature of Heating 0° C	Average Diffusion Current 100 Units = 3 µa.	Average Diameter of zone of inhibition mm.	Correlation Coefficient r
90	23.0	18	
100	23.0	18	
110	11.8	14	0.95*
120	1.8	5	
130	0.2	0	
140	0	0	95% = 0.878 99% = 0.959

* Significant at 95% level.

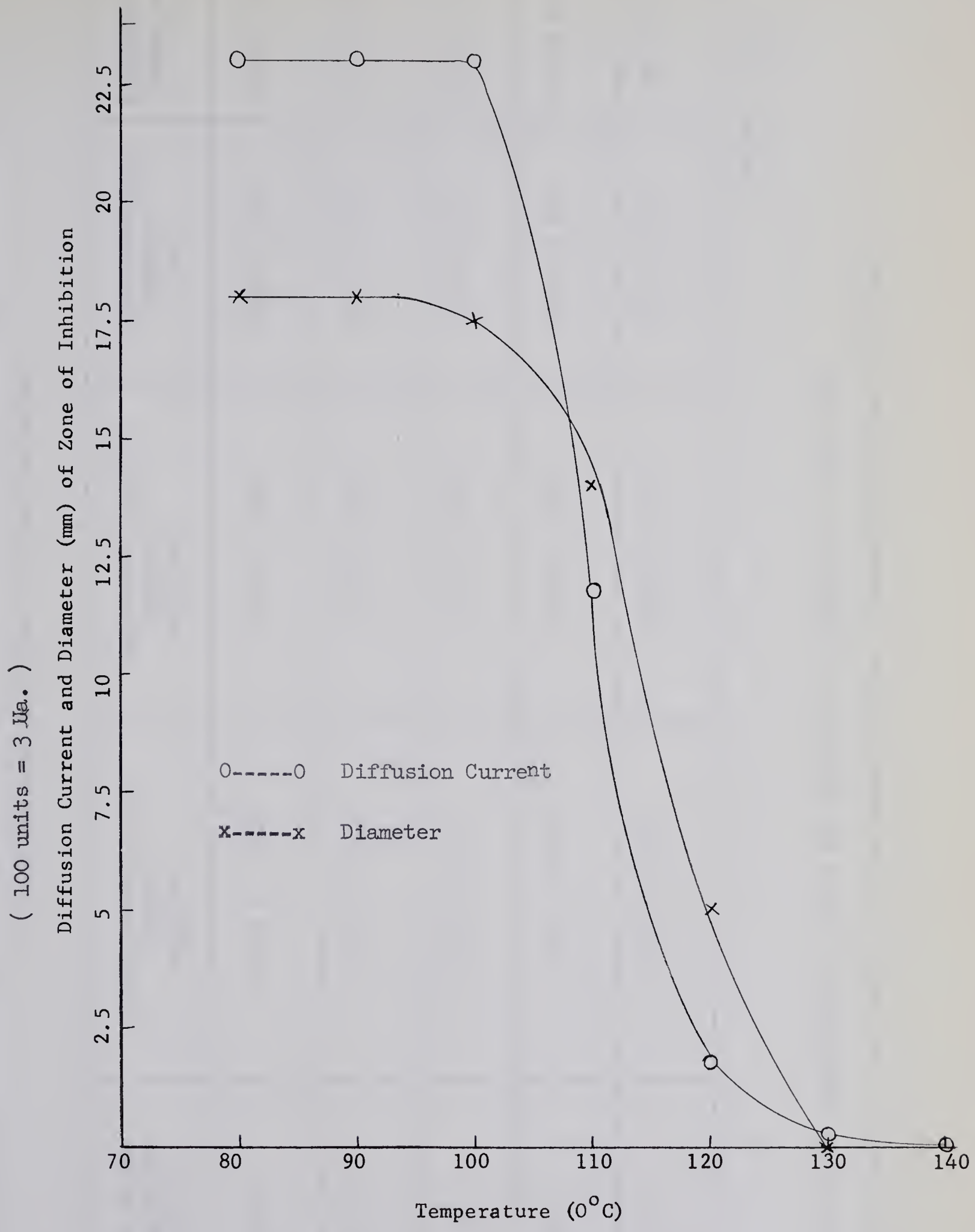


Figure 11. The comparison of microbiological and polarographic measurement of Oxytetracycline Amphoteric during degradation.



Table XLII

Results on the Polarographic Determination of the Concentration of Oxytetracycline Hydrochloride in Terramycin Capsule (Pfizer - 250 mg./capsule)

Amount of the preparation mg./10 ml.	Oxytetracycline HCl found mg./10 ml.	Equivalent Oxytetracycline mg./10 ml.	Oxytetracycline/ capsule mg.	% Stated Concentration
0.50	0.191 ± 0.004	0.177 ± 0.004	252.1 ± 5.7	100.8 ± 2.3
1.00	0.384 ± 0.011	0.356 ± 0.010	253.3 ± 7.2	101.3 ± 2.9
1.50	0.576 ± 0.014	0.534 ± 0.013	252.9 ± 6.1	101.2 ± 2.4
2.00	0.766 ± 0.018	0.709 ± 0.016	252.2 ± 5.8	100.9 ± 2.3
2.50	0.954 ± 0.020	0.884 ± 0.019	251.3 ± 5.3	100.5 ± 2.1

Average = 100.9
± 0.3

Individual experimental results are presented in Appendix I, Table XVII.

Each result is an average of five determinations ± Standard Deviation of the Mean.

Table XLIII

Results on the Polarographic Determination of the Recovery of Added Oxytetracycline Hydrochloride from Terramycin Capsule.

Amount of the preparation mg./10 ml.	Oxytetracycline HCl (98.2%)				
	Amount added mg.	Total present mg./10 ml.	Total found mg./10 ml.	Amound recovered mg.	% Recovery
0.75 mg.	0.125	0.418	0.418 ± 0.002	0.125 ± 0.002	100.0 ± 1.5
= 0.266 mg. Oxytetracycline	0.250	0.543	0.540 ± 0.006	0.248 ± 0.006	99.0 ± 2.5
= 0.287 mg. Oxytetracycline HCl	0.375	0.668	0.665 ± 0.008	0.373 ± 0.008	99.4 ± 2.1
≡ 0.293 mg. Oxytetracycline HC l (as 98.2%)	0.500	0.793	0.792 ± 0.009	0.499 ± 0.009	99.9 ± 1.8
	0.750	1.043	1.042 ± 0.023	0.749 ± 0.023	99.9 ± 3.0

Average = 99.7

Individual experimental results are presented in Appendix II, Table XVII.

Each result is an average of five determinations ± Standard Deviation of the Mean.

2. Terramycin I.V. Powder (Pfizer Corporation, Canada)

Each vial is stated to contain 250 mg. oxytetracycline HCl buffered with ascorbic acid.

In borate buffer of pH 9.75 ascorbic acid in concentration of 5.00 mg/ 10 ml and higher gave a very small wave at-0.90 v. and after that had an almost horizontal line up to the decomposition potential for the waves of the oxytetracycline HCl. The proportion in which ascorbic acid is present in the I.V. powder showed no effect on the residual current of the waves of oxytetracycline HCl since the measurement was carried out up to a concentration of 1.25 mg oxytetracycline HCl/ 10 ml. If measurement was intended for concentration higher than this level, a blank containing ascorbic acid had to be run for good quantitative results, which was difficult since the amount of ascorbic acid in the I.V. powder was not known.

The terramycin I.V. powder was completely soluble in water. The results are shown in Tables XLIV and XLV.

3. Terramycin I.M. solution (Pfizer Corporation, Canada)

Each ml of the two ml ampoules is stated to contain

Oxytetracycline	50 mg.
Lidocaine	2.00%
Magnesium chloride ($MgCl_2$, 6 H_2O)	2.50%
Sodium formaldehyde sulfoxylate	0.50%
Ethanolamine	1.70%
Propylene glycol	75.20%

Table XLIV

Results on the Polarographic Determination of the Concentration of Oxytetracycline Hydrochloride in Terramycin I.V. Powder (Pfizer - 250 mg./vial)

Amount of the preparation mg./10 ml.	Oxytetracycline HCl found mg./10 ml.	Oxytetracycline HCl/vial mg.	% Stated Concentration
1.25	0.216 ± 0.004	249.9 ± 4.5	100.0 ± 1.8
2.50	0.432 ± 0.009	249.9 ± 5.0	100.0 ± 2.0
3.75	0.643 ± 0.011	247.9 ± 4.4	99.2 ± 1.7
5.00	0.860 ± 0.017	248.9 ± 4.8	99.6 ± 1.9
7.50	1.287 ± 0.024	248.2 ± 4.6	99.3 ± 1.8

Average = 99.6 ± 0.4

Individual experimental results are presented in Appendix I, Table XVIII.

Each result is an average of five determinations ± Standard Deviation of the Mean.

Table XLV

Results on the Polarographic Determination of the Recovery of Added Oxytetracycline Hydrochloride from Terramycin I.V. Powder

Amount of the preparation mg./10 ml.	Oxytetracycline HCl (98.2%)				
	Amount added mg.	Total present mg./10 ml.	Total found mg./10 ml.	Amount recovered mg.	% Recovery
1.50 mg. = 0.258 mg. Oxytetracycline HCl = 0.263 mg. Oxytetracycline HCl (as 98.2%)	0.125	0.388	0.388 ± 0.002	0.125 ± 0.002	100.0 ± 1.6
	0.250	0.513	0.509 ± 0.005	0.246 ± 0.005	98.4 ± 2.0
	0.375	0.638	0.639 ± 0.009	0.376 ± 0.009	100.4 ± 2.5
	0.500	0.763	0.761 ± 0.008	0.498 ± 0.008	99.5 ± 1.6
	0.750	1.013	1.009 ± 0.019	0.746 ± 0.019	99.4 ± 2.5

Average = 99.6

Individual experimental results are presented in Appendix II, Table XVIII.

Each result is an average of five determinations ± Standard Deviation of the Mean.



Lidocaine at a concentration of 5.00 mg/10 ml showed a very small wave at -1.35 v i.e. in the region of the first wave of oxytetracycline HCl. However, the proportion in which it is present in the I. M. solution had no significant effect, since the analysis was limited mostly to low concentration of the antibiotic. None of the other components had any effect except magnesium chloride.

Magnesium chloride showed the same kind of effect on the waves of oxytetracycline as was encountered in the case of tetracycline HCl present in Tetrex I.M. (page 66). EDTA was a satisfactory chelating agent to remove the interfering effect of magnesium ion on the reduction waves of oxytetracycline HCl. Magnesium-EDTA did not have any effect on the original waves of the antibiotic.

A blank consisting of all the known components in the same proportion as are present in the pharmaceutical preparation was run to get a true measurement of the concentration of the antibiotic. Propylene glycol in the proportion in which it is present in the pharmaceutical preparation was added during the preparation of the standard solution of oxytetracycline HCl. Using approximately three times the equivalent amount of EDTA for the magnesium present in the samples, analysis was carried out in the usual way. The I. M. solution was diluted sufficiently with water to obtain a workable concentration of oxytetracycline HCl. The results are shown in Tables XLVI and XLVII.

Table XLVI

Results on the Polarographic Determination of the Concentration of Oxytetracycline Hydrochloride in Terramycin I.M. Solution (Pfizer - 100 mg./ 2 ml. ampoule)

Amount of the preparation ml./10 ml.	Oxytetracycline HCl found mg./10 ml.	Oxytetracycline HCl/ 2 ml. ampoule mg.	% Stated Concentration
0.010	0.502 ± 0.011	100.5 ± 2.3	100.5 ± 2.3
0.015	0.753 ± 0.013	100.4 ± 1.7	100.4 ± 1.7
0.020	0.993 ± 0.024	99.3 ± 2.4	99.3 ± 2.4
0.025	1.243 ± 0.024	99.4 ± 1.9	99.4 ± 1.9
0.030	1.704 ± 0.033	100.2 ± 1.9	100.2 ± 1.9

Average = 100.0 ± 0.6

Individual experimental results are presented in Appendix I, Table XIX.

Each result is an average of five determinations ± Standard Deviation of the Mean.

Table XLVII

Results on the Polarographic Determination of the Recovery of Added Oxytetracycline Hydrochloride from Terramycin I.M. Solution.

Amount of the preparation ml./10 ml.	Oxytetracycline HCl (98.2%)				
	Amount added mg.	Total present mg./10 ml.	Total found mg./10 ml.	Amount recovered mg.	% Recovery
0.005 ml. = 0.250 mg. Oxytetracycline = 0.270 mg. Oxytetracycline HCl = 0.275 mg. Oxytetracycline HCl (as 98.2%)	0.125	0.400	0.398 ± 0.002	0.124 ± 0.002	98.9 ± 2.0
	0.250	0.525	0.523 ± 0.007	0.249 ± 0.007	99.4 ± 3.0
	0.375	0.650	0.650 ± 0.008	0.375 ± 0.008	100.0 ± 2.0
	0.500	0.775	0.773 ± 0.012	0.499 ± 0.012	99.7 ± 2.4
	0.750	1.025	1.021 ± 0.010	0.747 ± 0.010	99.6 ± 1.4

Average = 99.6

Individual experimental results are presented in Appendix II, Table XIX.

Each result is an average of five determinations ± Standard Deviation of the Mean.

4. Terramycin Otic (Pfizer Corporation, Canada)

When prepared as directed in 5 ml of 90% propylene glycol, each ml is stated to contain

Oxytetracycline HCl		equivalent to 5 mg base
Polymyxin B sulphate	=	10,000 units equivalent to 1 mg. polymyxin B standard.
Benzocaine	=	50 mg.

The determination of potency could be carried out either on the dry powder or after preparing the solution as directed. The dry powder was preferred and was added to water in a volumetric flask to prepare the stock preparation.

In borate buffer of pH 9.75, benzocaine at a concentration of 15 mg./10 ml or higher, showed a very small gradual rise from -1.25 to -1.60 v and a small wave at -1.71 v. Polymyxin B sulphate in the same condition began to rise very slowly from -1.45 v. with a sharp rise at -1.65 v.

A blank, containing benzocaine and polymyxin B sulphate in the proportion in which they appear in the preparation was used for comparison. Measurement of diffusion current was limited to the first wave since there was a rise in the region of the second wave of oxytetracycline HCl. Polymyxin present in the pharmaceutical preparation could not be measured due to the interference from the second wave of oxytetracycline HCl and a high diffusion current due to

benzocaine in that region. The results are shown in Tables XLVIII and XLIX.

5. Terramycin Vaginal Tablets (Pfizer Corporation, Canada)

Each tablet is stated to contain

Oxytetracycline (as oxytetracycline HCl)	=	100 mg.
Polymyxin B standard (as polymyxin B sulphate)	=	10 mg.

The powder of the tablet was found to be not completely soluble in water. A few drops of 3 M HCl was added to the aqueous suspension to ensure complete solution of the oxytetracycline HCl.

Polymyxin B sulphate had the same effect on the waves of oxytetrcycline HCl as discussed under Terramycin Otic Solution and the measurement of the concentration of oxytetracycline HCl present in the tablet was done with reference to the first wave only. The results are shown in Tables L and LI.

(v) Calcium Diterramycin (Ca-dioxytetracycline)

Ca-diterramycin is a salt of one molecule of calcium and two molecules of oxytetracycline HCl. It is insoluble in water and was dissolved in water using 3 M HCl. Under similar conditions of pH and buffer to those used for oxytetracycline HCl, it gave waves at the same potentials but they were slightly different from those of oxytetracycline HCl. For the equivalent amount of oxytetracycline present in Ca-diterramycin and oxytetracycline HCl the first

Table XLVIII

Results of the Polarographic Determination of the Concentration of Oxytetracycline in Terramycin Otic (Pfizer - 25 mg./vial)

Amount of the preparation mg./10 ml.	Oxytetracycline HCl found mg./10 ml.	Equivalent Oxytetracycline mg./10 ml.	Oxytetracycline/ vial mg.	% Stated Concentration
2.00	0.196 ± 0.006	0.182 ± 0.006	24.7 ± 0.8	99.0 ± 3.0
4.00	0.391 ± 0.009	0.362 ± 0.008	24.6 ± 0.6	98.6 ± 2.2
6.00	0.591 ± 0.011	0.548 ± 0.010	24.8 ± 0.5	99.3 ± 1.8
8.00	0.780 ± 0.010	0.723 ± 0.009	24.6 ± 0.3	98.4 ± 1.2
10.00	0.980 ± 0.017	0.908 ± 0.016	24.7 ± 0.4	98.8 ± 1.7

Average = 98.8

Individual experimental results are presented in Appendix I, Table XX.

Each result is an average of five determinations ± Standard Deviation of the Mean.

Table XLIX

Results on the Polarographic Determination of the Recovery of Added Oxytetracycline Hydrochloride from Terramycin Otic.

Amount of the preparation mg./10 ml.	Oxytetracycline HCl (98.2%)				
	Amount added mg.	Total present mg./10 ml.	Total found mg./10 ml.	Amount recovered mg.	% Recovery
3.00 mg. = 272 mg. Oxytetracycline = 0.294 mg. Oxytetracycline HCl = 0.299 mg. Oxytetracycline HCl (as 98.2%)	0.125	0.424	0.425 ± 0.003	0.126 ± 0.003	100.5 ± 2.3
	0.250	0.549	0.548 ± 0.007	0.249 ± 0.007	99.6 ± 2.9
	0.375	0.674	0.671 ± 0.007	0.372 ± 0.007	99.2 ± 2.0
	0.500	0.799	0.802 ± 0.013	0.502 ± 0.013	100.5 ± 2.5
	0.750	1.049	1.054 ± 0.014	0.754 ± 0.014	100.6 ± 1.8

Average = 100.0

Individual experimental results are presented in Appendix II, Table XX.

Each result is an average of five determinations ± Standard Deviation of the Mean.

Table L

Results on the Polarographic Determinations of the Concentration of Oxytetracycline in Terramycin Vaginal Tablet (Pfizer - 100 mg/tablet).

Amount of the preparation mg./10 ml.	Oxytetracycline HCl found mg./10 ml.	Equivalent Oxytetracycline mg./10 ml.	Oxytetracycline/ tablet mg.	% Stated Concentration
1.25	0.096 ± 0.002	0.089 ± 0.002	94.2 ± 2.1	94.2 ± 2.1
2.50	0.195 ± 0.006	0.180 ± 0.006	95.1 ± 2.9	95.1 ± 2.9
5.00	0.390 ± 0.010	0.362 ± 0.010	95.3 ± 2.5	95.3 ± 2.5
7.50	0.586 ± 0.010	0.543 ± 0.009	95.3 ± 2.5	95.3 ± 2.5
10.00	0.780 ± 0.016	0.723 ± 0.015	95.3 ± 2.0	95.3 ± 2.0

Average = 95.0 ± 0.4

Individual experimental results are presented in Appendix I, Table XXI.

Each result is an average of five determinations ± Standard Deviation of the Mean.

Table LI

Results on the Polarographic Determination of the Recovery of Added Oxytetracycline Hydrochloride from Terramycin Vaginal Tablet.

Amount of the preparation mg./10 ml.	Oxytetracycline HCl (98.2%)				
	Amount added mg.	Total present mg./10 ml.	Total found mg./10 ml.	Amount recovered mg.	% Recovery
3.00 mg. = 0.216 mg. Oxytetracycline = 0.233 mg. Oxytetracycline HCl = 0.238 mg. Oxytetracycline HCl (as 98.2%)	0.125	0.362	0.362 ± 0.002	0.124 ± 0.002	99.3 ± 2.0
	0.250	0.488	0.488 ± 0.006	0.250 ± 0.006	100.0 ± 2.4
	0.375	0.612	0.611 ± 0.007	0.373 ± 0.007	99.5 ± 2.0
	0.500	0.738	0.734 ± 0.011	0.496 ± 0.011	99.3 ± 2.2
	0.750	0.988	0.984 ± 0.011	0.746 ± 0.011	99.5 ± 1.5

Average = 99.5

Individual experimental results are presented in Appendix II, Table XXI.

Each Result is an average of five determinations ± Standard Deviation of the Mean.

wave in the former was slightly smaller and the second slightly larger than the waves observed for oxytetracycline HCl alone.

Carrying out polarographic analysis for calcium chloride it was found that the calcium ion gave an E 1/2 at much more negative potential under the conditions used for antibiotic determination. But when it was added to oxytetracycline HCl, it influenced the waves of the antibiotic. When EDTA was added to this mixture the first wave began to increase and the second wave decreased until with equimolecular concentration of calcium ion and EDTA the waves became identical with those of the pure oxytetracycline HCl. Under this condition Ca-diterramycin behaved in an identical manner to pure oxytetracycline HCl. Using approximately three times the equivalent concentration of EDTA than the amount of calcium present, the analysis was carried out in borate buffer at pH 9.75 as in the case of oxytetracycline HCl. The results are shown in Table LII and Figure 12. The result of the microbiological comparison are shown in Table LIII and Figure 13.

(v)a. Pharmaceutical Preparations

1. Terramycin Pediatric Drops (Pfizer Corporation, Canada)

Each ml of the preparation is stated to contain

Ca-diterramycin equivalent to	100 mg. oxytetracycline
N-acetyl glucosamine equivalent to	100 mg. glucosamine HCl

N-acetyl glucosamine like glucosamine HCl did not have any wave in the region of oxytetracycline HCl. The heavy suspension was

Table LII

Data on the Diffusion Currents of the First Wave of Ca-Diterramycin in Borate Buffer at pH 9.75 ± 0.05 and EDTA. $m^{2/3}t^{1/6} = 1.65$ mg.$^{2/3}$Sec.$^{-1/2}$

Concentration mg/10 ml.	Diffusion Current 100 units = 3 μa	Average ± S.D.M.
0.050	2.00 2.00 2.00 2.17 2.00	2.0 ± 0.1
0.125	4.00 4.00 4.00 4.00 4.25	4.0 ± 0.1
0.250	7.00 7.25 7.00 7.25 7.00	7.1 ± 0.1
0.375	10.25 10.00 10.50 10.25 10.25	10.2 ± 0.2
0.500	14.00 13.75 14.50 14.00 13.75	14.0 ± 0.3
0.750	20.25 20.50 20.75 20.00 19.75	20.2 ± 0.4
1.000	26.50 25.75 27.25 26.50 27.00	26.6 ± 0.5
1.250	33.50 34.00 32.25 33.00 32.75	33.5 ± 0.7

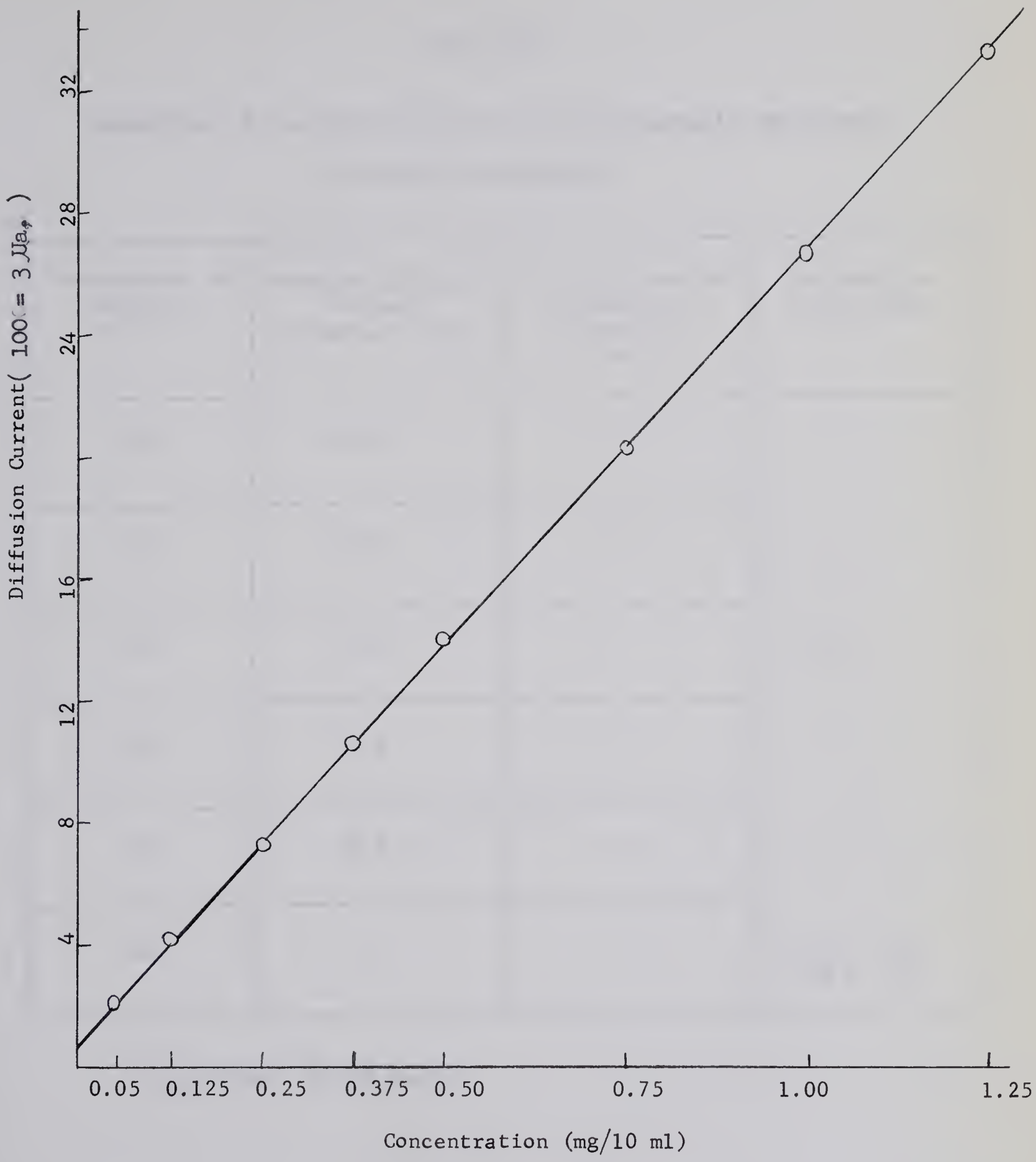


Figure 12. The graph of Calcium Diterramycin standard.

Table LIII

Comparison of the Microbiological and Polarographic Measurement of Calcium Diterramycin.

Temperature of Heating 0^{o} C	Average Diffusion Current 100 Units = 3 µa.	Average Diameter of zone of inhibition mm.	Correlation Coefficient r
90	22.0	18	
100	22.0	18	
110	19.0	18	0.97*
120	4.5	12	
130	2.0	8	
140	0	0	95% = 0.95 99% = 0.99

* Significant at 95% level.

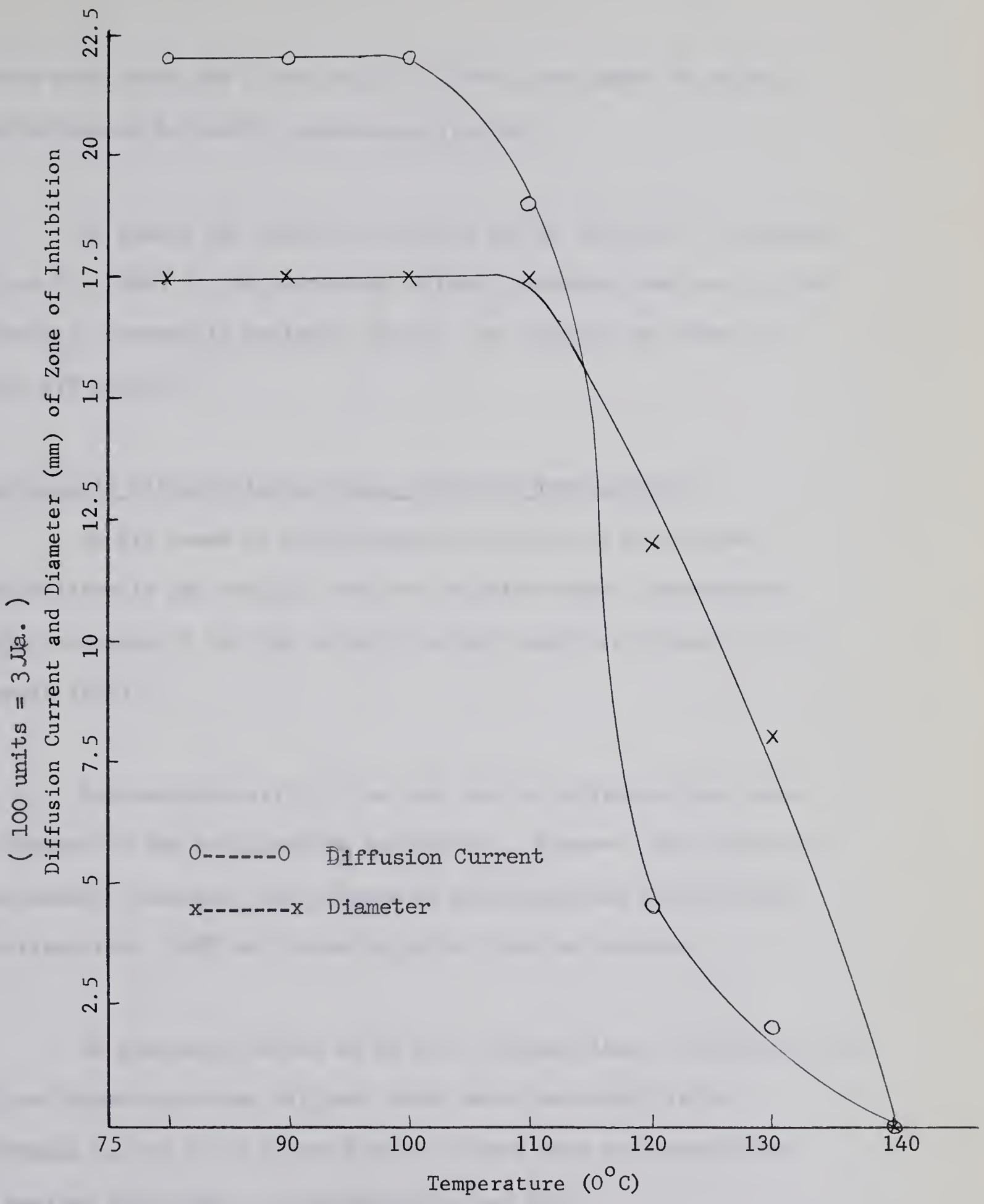


Figure 13. The comparison of microbiological and polarographic measurement of Calcium Diterramycin during degradation.

diluted with water and a few drops of 3 M HCl was added to convert Ca-diterramycin to soluble oxytetracycline HCl.

To remove the effect of calcium ion on the waves of oxytetracycline HCl, EDTA in the proportion already mentioned was used in the analysis of terramycin pediatric drops. The results are shown in Tables LIV and LV.

(vi) Polarographic Differentiation Among Different Tetracyclines

In all cases of polarographic reduction of the various tetracyclines it was thought that the two major waves corresponded to the reduction of the two carbonyl groups which are present in the molecule (136).

Polarographically, it was not easy to differentiate among the members of the tetracycline antibiotics. However, the following polarographic behaviour was helpful in distinguishing tetracycline, Cl-tetracycline, DMCT and oxytetracycline from one another.

In phosphate buffer at pH 6.00, tetracycline, Cl-tetracycline, DMCT and oxytetracycline all gave three waves and with little difference in the E 1/2 values but the second wave of tetracycline was smaller than that of Cl-tetracycline and DMCT.

In the same buffer, at pH 6.75, DMCT gave only two waves. The second wave of the three waves of tetracycline was absent in DMCT and

Table LIV

Results on the Polarographic Determination of the Concentration of Oxytetracycline in Terramycin Pediatric Drops (Pfizer - 100 mg./ml.)

Amount of the preparation ml./10 ml.	Ca-Ditteramycin found mg./10 ml.	Equivalent Oxytetracycline mg./10 ml.	Oxytetracycline/ ml. mg.	% Stated Concentration
0.00125	0.131 ± 0.004	0.125 ± 0.004	100.2 ± 3.0	100.2 ± 3.0
0.00250	0.263 ± 0.004	0.252 ± 0.004	100.8 ± 1.6	100.8 ± 1.6
0.00375	0.395 ± 0.009	0.379 ± 0.008	101.0 ± 2.3	101.0 ± 2.3
0.00500	0.526 ± 0.009	0.504 ± 0.009	100.8 ± 1.7	100.8 ± 1.7
0.00750	0.791 ± 0.017	0.758 ± 0.016	101.0 ± 2.2	101.0 ± 2.2

Average = 100.8 ± 0.3

Individual experimental results are presented in Appendix I, Table XXII.

Each result is an average of five determinations ± Standard Deviation of the Mean.

Table LV

Results on the Polarographic Determination of the Recovery of Added Oxytetracycline Hydrochloride from Terramycin Pediatric Drops.

Amount of the preparation ml./10 ml.	Calcium Diterramycin (87.1%)				
	Amount added mg.	Total present mg./10 ml.	Total found mg./10 ml.	Amount recovered mg.	% Recovery
0.0025 ml. = 0.252 mg. Oxytetracycline = 0.263 mg. Ca-Diterramycin = 0.302 mg. Ca-Diterramycin (as 87.1%)	0.125	0.427	0.427 ± 0.002	0.125 ± 0.002	100.0 ± 2.0
	0.250	0.552	0.550 ± 0.007	0.248 ± 0.007	99.4 ± 3.0
	0.375	0.677	0.675 ± 0.008	0.373 ± 0.008	99.6 ± 2.0
	0.500	0.802	0.798 ± 0.012	0.497 ± 0.012	99.3 ± 2.4
	0.750	1.052	1.045 ± 0.012	0.743 ± 0.012	99.1 ± 1.6

Average = 99.5

Individual experimental results are presented in Appendix II, Table XXII.

Each result is an average of five determinations ± Standard Deviation of the Mean.

although present was insignificant in Cl-tetracycline at this pH. Oxytetracycline gave three poorly developed waves under this condition.

At pH 7.25, tetracycline gave a very small second wave like the second wave of Cl-tetracycline at pH 6.75 and both Cl-tetracycline and DMCT gave two waves only. Oxytetracycline still gave three poorly resolved and ill-developed waves.

At pH 7.75, all the antibiotics gave only two waves. Tetracycline, Cl-tetracycline and DMCT gave similar waves whereas the waves of oxytetracycline were quite different. From pH 8.50 onwards all of them showed the same polarographic waves.

In borate buffer at pH 7.75, there was found to be a slight difference in the two waves of tetracycline, Cl-tetracycline and DMCT. Tetracycline and Cl-tetracycline gave two waves at the same potentials, but the two waves in the case of the latter were not significantly separated. The two waves of DMCT were obtained at slightly less negative potentials. The E 1/2 values for the two waves were -1.17 and -1.40 v for tetracycline and -1.05 and 1.35 v for the DMCT.

II. Streptomycins

(i) Streptomycin Sulphate

Levy, Schwed and Sackett (137) in 1946 did the first polarographic work on streptomycin. They developed the first polarographic method for determining streptomycin using the pH range of 13.60 - 13.80 with tetramethyl ammonium hydroxide as the base electrolyte. Bricker and Vail (138) used this polarographic method to study the alkaline decomposition of this antibiotic. Heuser, Dolliver and Stiller (139) used the polarographic method for studying the tautomeric changes of crystalline streptomycin salts from the difference in the forms of the wave.

An amperometric method for determining both streptomycin and dihydrostreptomycin was developed by Conn and Norman (140) which was based on the formation of a water insoluble compound of these two antibiotics with certain anion dyes.

Goodey, Couling and Hart (141) used the polarographic method for the determination of streptomycin in fermentation broth to check the yield of production of the antibiotic. da Cunha (142) reported in 1963 a polarographic method for the analysis of a mixture of streptomycin and dihydrostreptomycin. According to this method the determination of streptomycin was followed in the same way as described earlier using 0.1^{N} sodium hydroxide. Dihydrostreptomycin measurement, however, was indirect. He used two such methods for

its determination. The first was based on the lowering of polarographic maxima of the cobalt wave by dihydrostreptomycin and the second was based on the measurement of formaldehyde produced by oxidation of this antibiotic with periodate (cf. Garloch and Grove, J. Clin. Invest. 28,843,1949).

The polarographic behaviour of streptomycin in the present work is summarized as follows:

In the acid pH range there were several irregular small waves. In the alkaline region from pH 8.50 there were two well defined adjacent waves in which the second wave was not very sharp. At pH 10 the second wave had the best shape and after that point it began to lose its shape until at pH 12.25 it completely vanished and there was only one wave. At pH 13.45 this single wave was most suitable for the measurement of diffusion current (Figure 14).

At such high pH, a suitable buffer was difficult to obtain. The use of alkalis like sodium hydroxide or tetraethyl ammonium hydroxide was found necessary and tetraethyl ammonium hydroxide was preferred as the antibiotic was more stable in it than sodium hydroxide (138). The E 1/2 value for the single wave was -1.61 ± 0.01 v. The results are shown in Table LVI and Figure 15. The results of the microbiological comparison are shown in Table LVII and Figure 16.

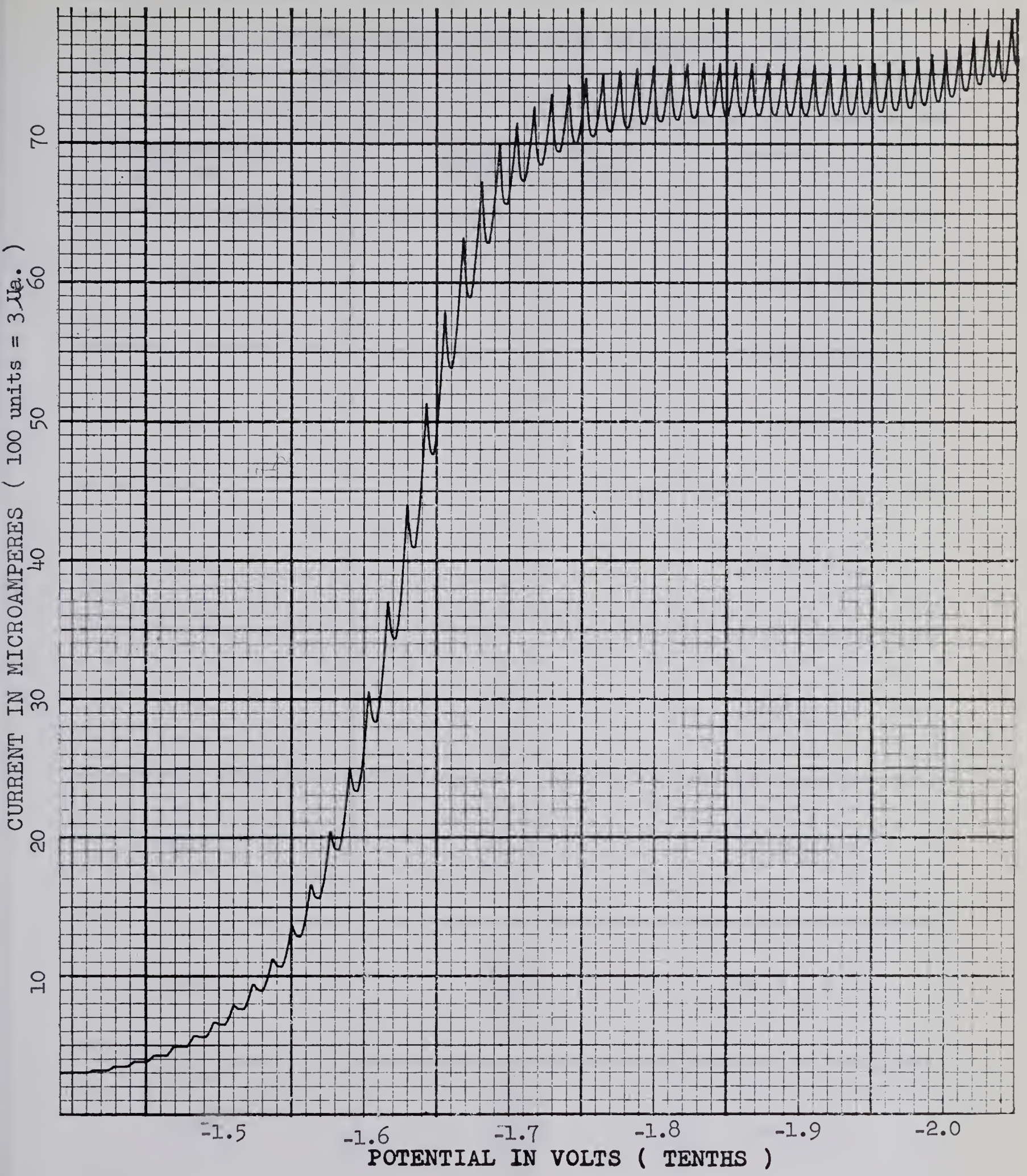


FIGURE 14. THE CURRENT-VOLTAGE WAVE OF STREPTOMYCIN SULPHATE

Table LVI

Data on the Diffusion Currents of the Single Wave of Streptomycin Sulphate in Tetraethyl Ammonium Hydroxide at pH 13.45 ± 0.05

$m^{2/3} t^{1/6} = 1.61$ mg. $^{2/3}$ Sec. $^{-1/2}$

Concentration mg/10 ml.	Diffusion Current 100 units = 3 Ua.	Average ± S.D.M.
0.050	1.75 1.67 1.75 1.75 1.75	1.7 ± 0.0
0.125	4.25 4.25 4.25 4.25 4.50	4.3 ± 0.1
0.250	8.50 8.25 8.75 8.50 8.50	8.5 ± 0.2
0.375	13.00 12.75 12.75 12.50 12.75	12.8 ± 0.2
0.500	17.50 17.00 16.50 16.75 17.25	17.0 ± 0.3
0.750	25.50 25.00 25.25 26.00 25.50	25.4 ± 0.4
1.000	34.00 34.50 33.50 33.75 34.50	34.0 ± 0.4
1.250	42.08 42.50 43.33 41.25 42.92	42.4 ± 0.8

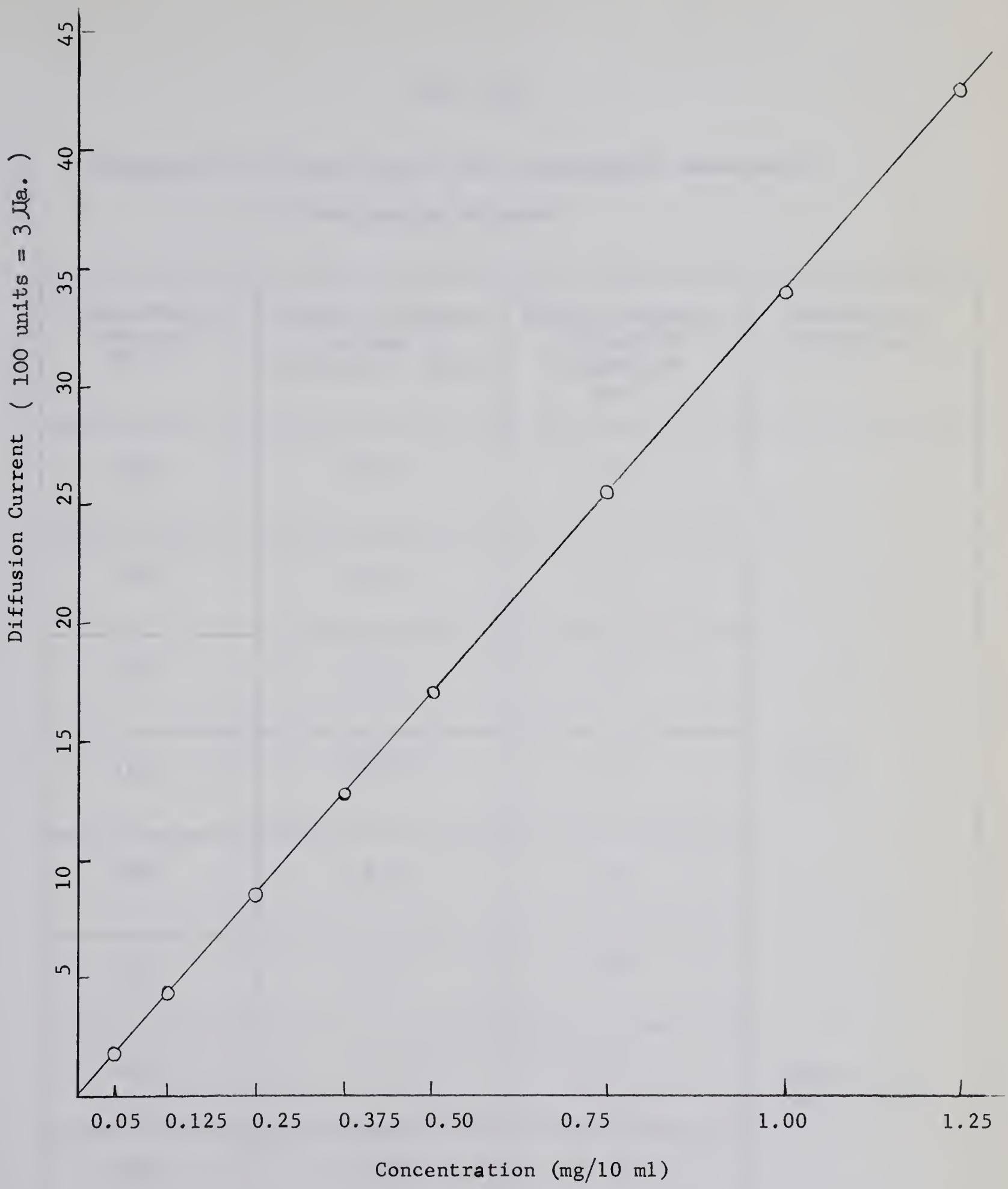


Figure 15. The graph of Streptomycin Sulphate standard.

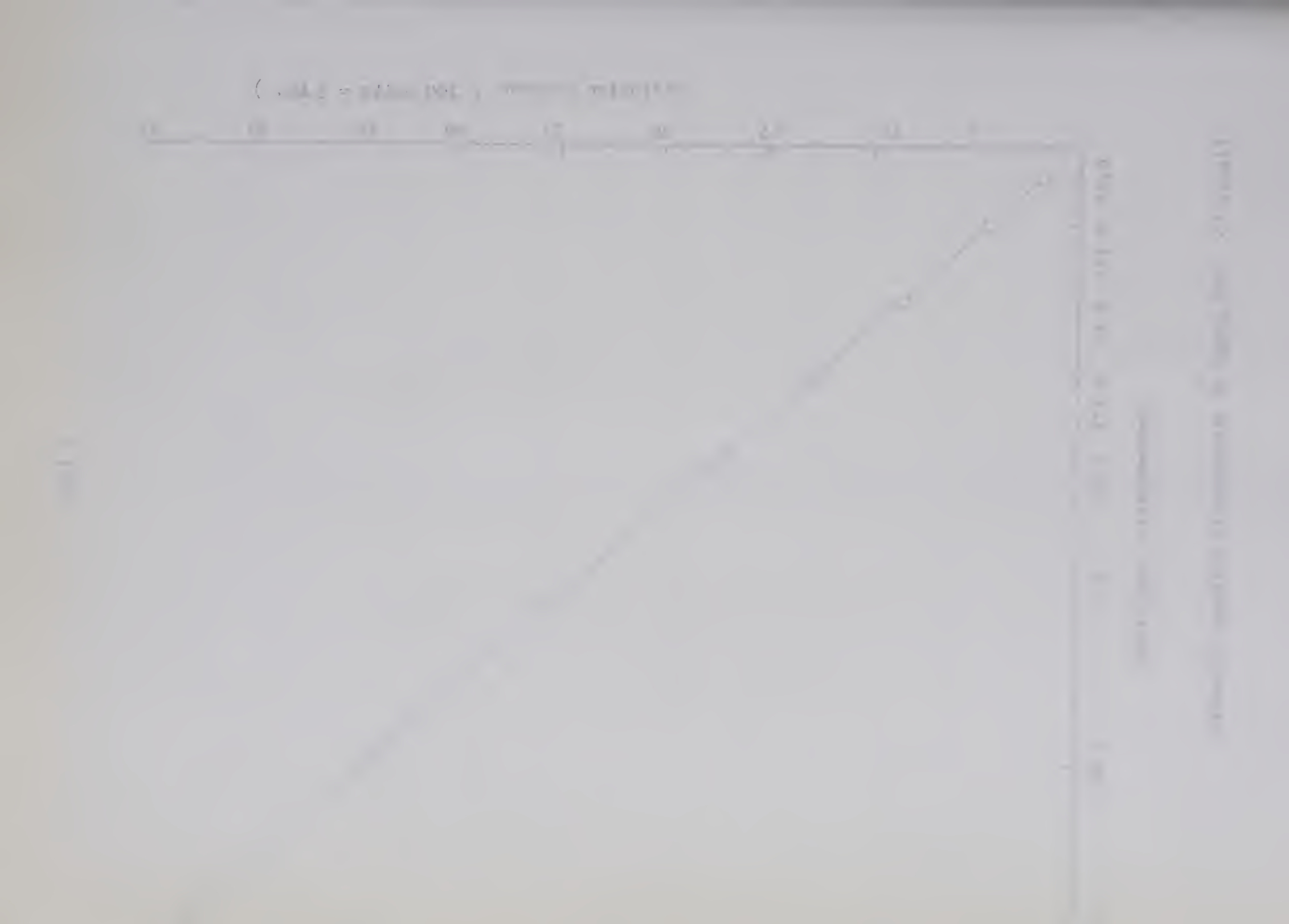


Table LVII

Comparison of Microbiological and Polarographic Measurement of Streptomycin Sulphate.

Temperature of heating 0^{o} C	Average Diffusion current 100 Units = 3 μa.	Average Diameter of zone of inhibition mm.	Correlation Coefficient r
80	18.0	17	
90	18.0	17	
100	17.2	16	
110	16.0	16	0.98*
120	13.0	14	
130	10.0	12	
140	7.0	9	95% = 0.754 99% = 0.874
150	4.5	7	

* Significant at 99% level.

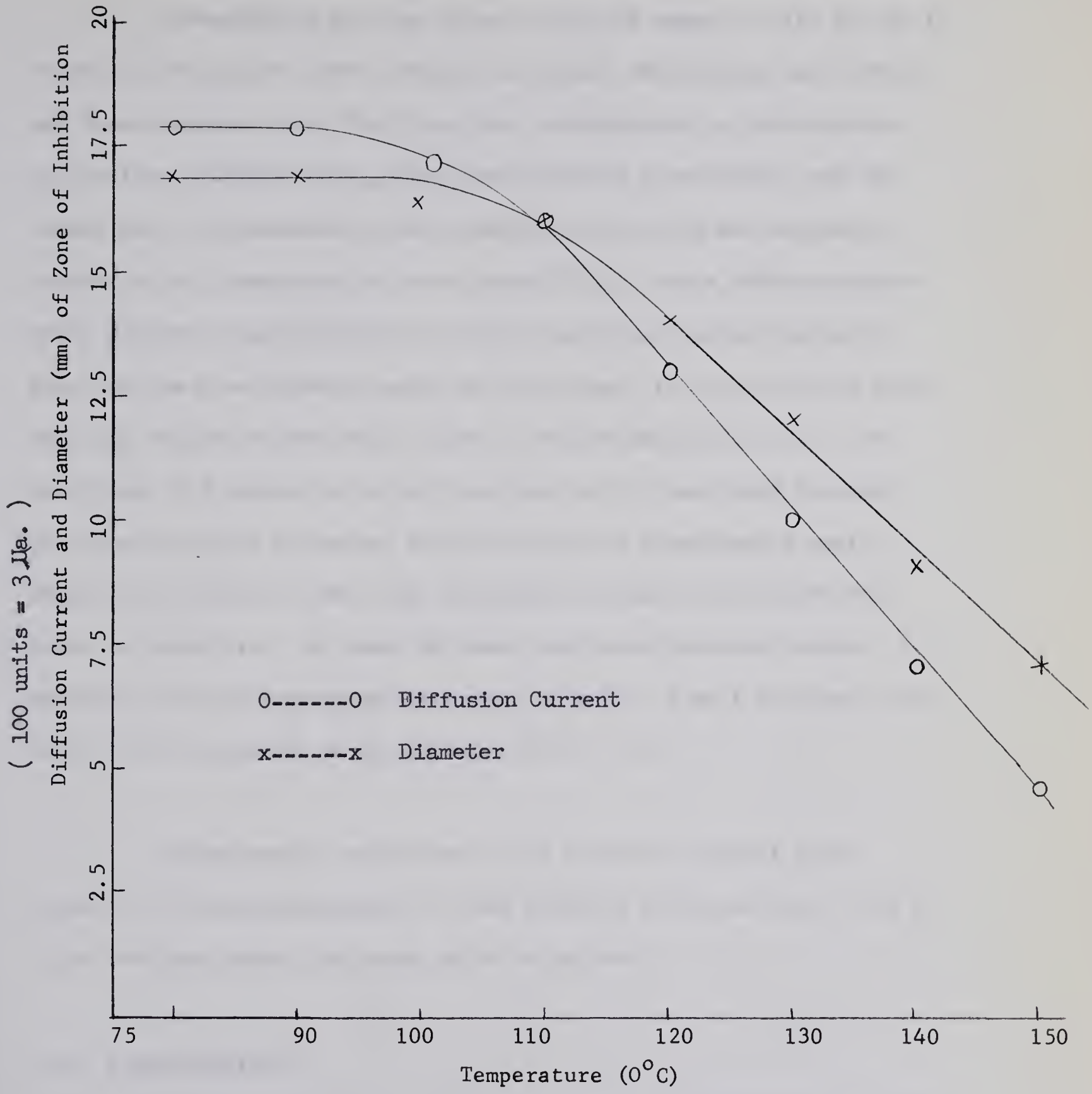


Figure 16. The comparison of microbiological and polarographic measurement of Streptomycin Sulphate during degradation.

Streptomycin gave two waves in the pH range 8 - 12, and it is known that these two waves combined at higher temperature and Brezina and Zuman surmised that the first wave corresponded to the reduction of the free aldehyde group, which was hydrated in solution, and the second wave corresponded to the aldehyde group which was partially blocked with a guanidine or amino group (143). Since dihydrostreptomycin differs from streptomycin only in having an alcohol group in place of the free aldehyde group of the latter, it should follow that only one reduction wave would occur in the pH range of 8 - 12. In fact there did appear to be only one wave but it was found to occur at a more negative potential than the waves of streptomycin would suggest and before it was fully developed the base electrolyte wave began to interfere. At lower pH there were some irregular waves. A variety of base electrolytes were used in vain. A well developed wave could not be produced in the pH range of 8 - 12.

Polarographic oxidation of the secondary alcohol group present in dihydrostreptomycin to the aldehyde group was also tried in a few ordinary media, but again with no success.

(ii) Streptohydrazid

Streptohydrazid is a complex mixture of streptomycin sulphate and isoniazid. From pH 3 - 11, two large polarographic waves occurred which were very close to one another and these were followed by a small third wave. From pH 11 - 12 there were only two waves but the peak of

the first wave was nearly masked by the smaller wave. Beyond pH 12 a single wave was obtained. From the preliminary studies of E 1/2 values of the waves of streptomycin sulphate, isoniazid and streptohydrazid under identical conditions of pH etc., it seemed that where there were two waves obtained with streptohydrazid the first wave was due to the isoniazid part and the second wave was due to the streptomycin moiety. A mixture of streptomycin sulphate and isoniazid in the proportion in which they are present in streptohydrazid, was found to give waves whose diffusion currents were correspondingly less than those of the waves for the same amounts of these two components separately. And at pH above 12.00 where the mixture gave two waves the second wave gradually diminished with time until after about 20 - 24 hours it completely disappeared and the single wave corresponded identically with the single wave of a fresh equimolar concentration of streptohydrazid. It thus appeared that some polarographically active groups of streptomycin were involved in the formation of the streptohydrazid complex.

Since streptohydrazid gave a single wave above pH 12.00 and the two waves that were obtained below pH 11.00 were not separated from one another, pH 13.45 with tetraethyl ammonium hydroxide was most suitable for quantitative measurement of streptohydrazid. The E 1/2 value for the single wave was -1.41 ± 0.01 v. The results are shown in Table LVIII and Figure 17. Microbiological comparisons are presented in Table LIX and Figure 18.

Table LVIII

Data on the Diffusion Currents of the Single Wave of Streptohydrazid in Tetraethyl Ammonium Hydroxide at pH 13.45 ± 0.05

$m^{2/3} t^{1/6} = 1.62$ mg. $^{2/3}$ Sec. $^{-1/2}$

Concentration mg./10 ml.	Diffusion Current 100 units = 3 µa.	Average ± S.D.M.
0.050	2.37 2.25 2.25 2.25 2.25	2.3 ± 0.0
0.125	4.75 4.75 4.50 4.75 4.75	4.7 ± 0.1
0.250	9.00 9.25 8.75 9.00 9.00	9.0 ± 0.2
0.375	12.75 12.50 12.75 13.00 12.75	12.8 ± 0.2
0.500	17.50 16.75 16.75 17.25 17.00	17.0 ± 0.3
0.750	25.75 24.50 25.50 25.25 25.25	25.2 ± 0.5
1.000	33.50 32.75 34.00 33.00 34.50	33.5 ± 0.6
1.250	41.00 42.50 40.50 42.00 41.50	41.5 ± 0.8

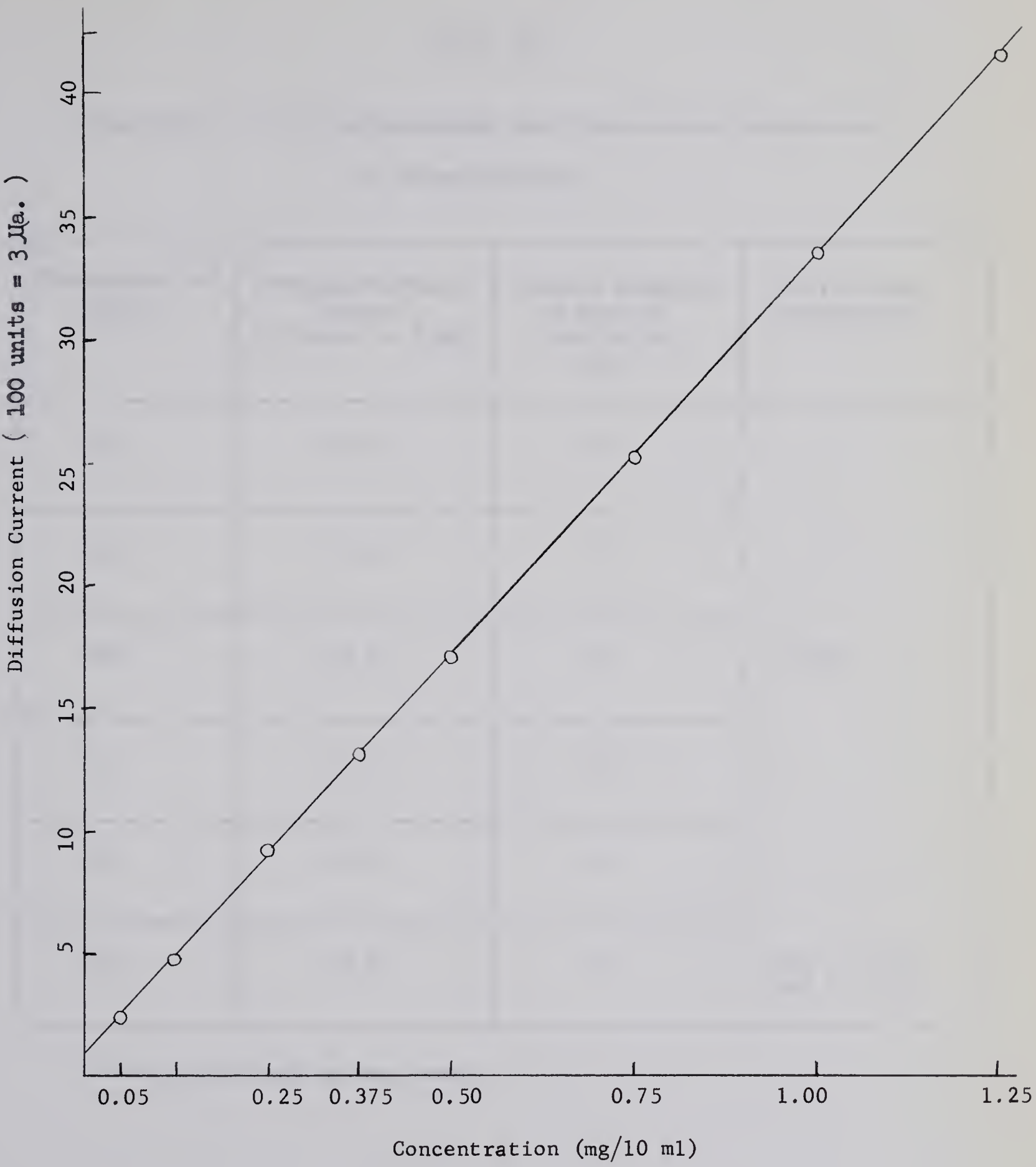


Figure 17. The graph of Streptohydrazid standard.

Table LIX

Comparison of the Microbiological and Polarographic Measurement of Streptohydrazid

Temperature of heating 0^{o} C	Average Diffusion Current 100 Units = 3 Ua.	Average Diameter of zone of inihibition mm.	Correlation Coefficient r
100	22.0	16	
110	21.0	16	
120	21.0	16	0.95*
130	21.0	16	
140	20.0	14	
150	16.0	14	95% = 0.997 99% = 1.000

* Not significant at any level.

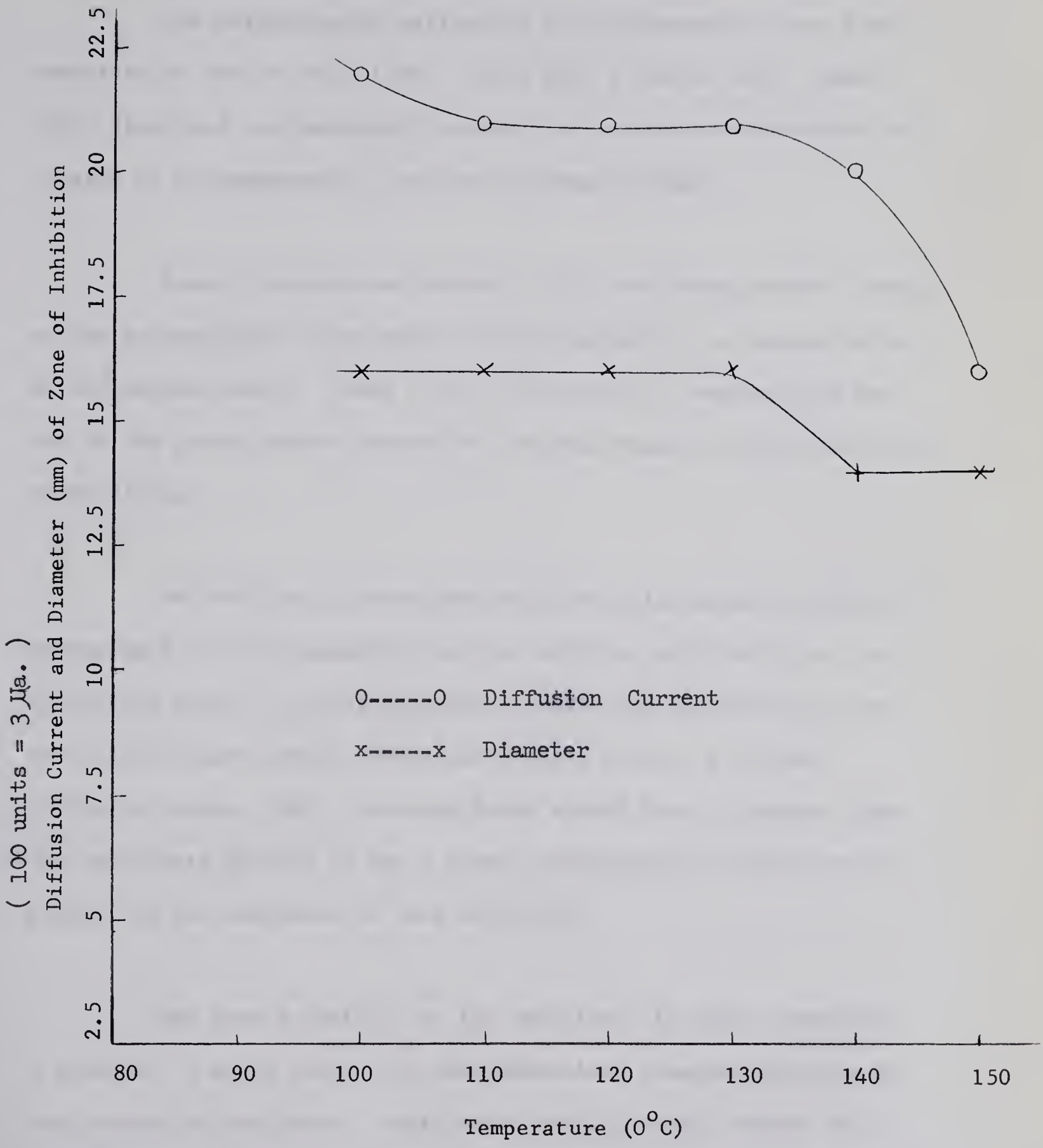


Figure 18. The comparison of microbiological and polarographic measurement of Streptohydrazid during degradation.

III (i) Chloramphenicol

The polarographic estimation of chloramphenicol was first described in 1950 by Hess (144). More than a decade later, Lanza (145) described a polarographic method for continuous measurement of eluates of chloramphenicol from ion exchange columns.

Russu, Cruceanu and Barcaru (146) have shown another example of the polarographic measurement of chloramphenicol in suppositories in non-aqueous media. Summa (147) very recently, demonstrated the use of the polarographic method for the measurement of chloramphenicol preparations.

One difficulty associated with the polarographic method of measurement of chloramphenicol is that both the antibiotic and its hydrolysis product, 1-p-nitrophenyl-2-amino-1,3,-propanediol, give well defined waves which correspond closely both in E 1/2 and diffusion current (144). However, Summa stated that it appears that the hydrolysis product is not a normal decomposition product or by-product in the synthesis of this antibiotic.

The poor solubility of the antibiotic in water presented a problem. A major portion of chloramphenicol remained floating on the surface of the water. Addition of acid or alkali caused only partial improvement in the solubility. To obtain a complete solution of the antibiotic it was first dissolved in 10% alcohol. The concentration of alcohol after subsequent dilution with water and buffer for analysis was not more than 0.50%.

From pH 3 to 4.50, chloramphenicol gave three reduction waves and from pH 5.00 to 10.00 always gave two waves which changed their form gradually with increased pH but the diffusion current remained fairly constant. Any of the phosphate, borate or biphthalate buffers could be used. Acetate buffer was not suitable since the buffer gave a small wave immediately before the waves of the antibiotic although, larger waves of chloramphenicol were obtained in this buffer. In both phosphate and borate buffer, the residual current was found to increase slowly. Biphthalate buffer gave a much better residual current and was the buffer of choice. The most suitable pH was found to be 5.20 ± 0.05. A maximum appeared after the first wave and a few drops of 0.1% gelatin were used to suppress the maximum. The first wave was found to be much better than the second wave for quantitative measurements of diffusion current. The E 1/2 values for the two waves at this pH in biphthalate buffer were 0.345 ± 0.005 v & -0.72 ± 0.01 v. The results are shown in Table LX and Figure 19.

(i)a Pharmaceutical Preparations

1. Chloromycetin Ophthalmic (Parke Davis and Co.)

Each vial is stated to contain 25 mg. chloramphenicol and borate buffer equivalent to 100 mg. boric acid.

Borate buffer itself did not cause a wave in the region of the waves of chloramphenicol. The addition of borate buffer to the solution of the antibiotic did not interferewith the waves of the antibiotic when analysed in biphthalate buffer. Boric acid in the same

Table IX

Data on the Diffusion Currents of Chloramphenicol in Biphthalate Buffer at pH 5.20 ± 0.05. $m^{2/3} t^{1/6} = 1.70$ mg. $^{2/3}$ Sec. $^{-1/2}$

Concentration mg./10 ml.	Diffusion Current 100 units = 3 μa.	Average ± S.D.M.
0.025	2.17 2.00 2.23 2.00 2.10	2.1 ± 0.1
0.05	4.00 4.00 4.17 4.08 3.95	4.0 ± 0.1
0.125	10.25 10.25 10.00 9.75 9.75	10.0 ± 0.2
0.25	21.00 19.75 20.00 20.25 20.25	20.2 ± 0.5
0.375	31.00 30.25 29.25 29.75 30.75	30.2 ± 0.7
0.50	39.00 41.00 40.25 39.25 39 75	39.8 ± 0.8
0.75	62.08 60.00 59.58 60.00 59.58	60.2 ± 1.0
1.00	80.00 80.42 79.17 81.25 77.92	79.8 ± 1.3

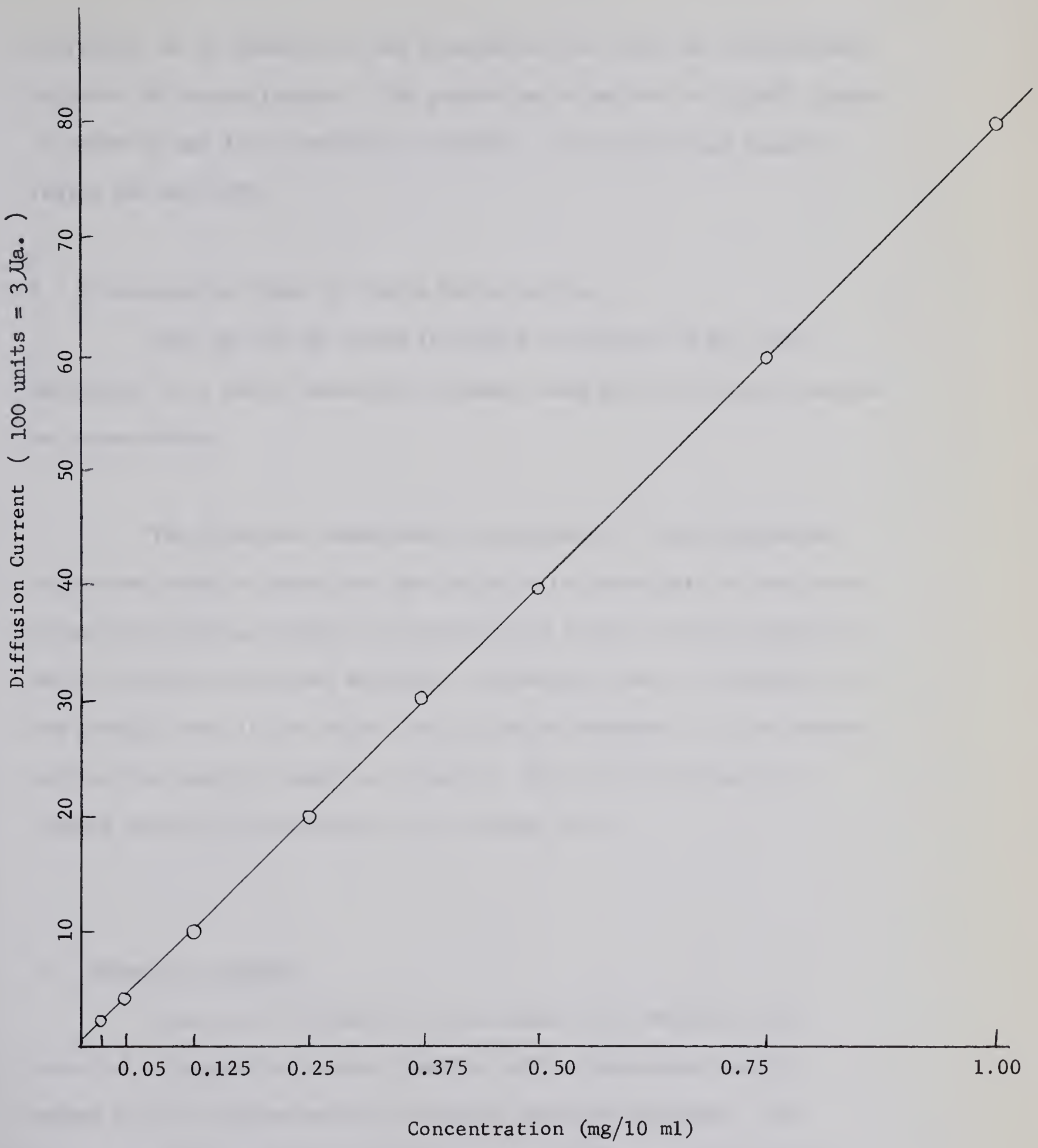


Figure 19. The graph of Chloramphenicol standard.

proportion as is present in the preparation was added to the standard solution of the antibiotic. The powder was dissolved in 10.00% alcohol in which it was found completely soluble. The results are shown in Tables LXI and LXII.

2. Chloromycetin Cream 1% (Parke Davis and Co.)

Each gm. of the cream is stated to contain 10 mg. chloramphenicol in a water immiscible ointment base with 0.1% propyl paraben as preservative.

The cream was dissolved in isopropanol. When biphthalate buffer was added to carry out the analysis the materials of the cream became insoluble and there was considerable foaming during deaeration which interfered with the analysis. Although it was not attempted, it was thought that if the buffer could also be prepared in a non-aqueous solvent the analysis might be possible. This would eliminate the lengthy extraction procedure used by Summa (147).

IV. Neomycin Sulphate

Bulant and Parizkova in their paper (70) mentioned the use of a polarographic method together with a spectrophotometric method for the determination of neomycin methanesulphonate. The method was to bring about the decomposition of the salt with mineral acid and the formaldehyde thus formed was measured after it was separated by distillation.

Table LXI

Results on the Polarographic Determination of the Concentration of Chloramphenicol in Chloromycetin Ophthalmic (Parke Davis - 25 mg./vial)

Amount of the prepatation mg./10 ml.	Chloramphenicol found mg./10 ml.	Chloramphenicol/vial mg.	% Stated Concentration
1.00	0.201 ± 0.004	25.4 ± 0.4	101.8 ± 1.8
2.00	0.399 ± 0.009	25.3 ± 0.6	101.0 ± 2.2
3.00	0.601 ± 0.009	25.3 ± 0.4	101.3 ± 1.6
4.00	0.802 ± 0.013	25.4 ± 0.4	101.5 ± 1.7
5.00	1.005 ± 0.019	25.4 ± 0.5	101.7 ± 2.0

Average = 101.5

Individual experimental results are presented in Appendix I, Table XXIII.

Each result is an average of five Determinations ± Standard Deviation of the Mean.



Table LXII

Results on the Polarographic Determination of the Recovery of Added Chloramphenicol from Chloromycetin Ophthalmic.

Amount of the preparation mg./10 ml.	Chloramphenicol				
	Amount added mg.	Total present mg./10 ml.	Total found mg./10 ml.	Amount recovered mg.	% Recovery
1.0 mg. = 0.201 mg. Chloramphenicol	0.125	0.326	0.325 ± 0.002	0.125 ± 0.002	100.0 ± 1.9
	0.250	0.451	0.451 ± 0.004	0.251 ± 0.004	100.3 ± 1.4
	0.375	0.576	0.574 ± 0.008	0.374 ± 0.008	99.7 ± 2.2
	0.500	0.701	0.699 ± 0.010	0.499 ± 0.010	99.7 ± 1.9
	0.625	0.826	0.826 ± 0.011	0.626 ± 0.011	100.1 ± 1.7

Average = 99.9

Individual experimental results are presented in Appendix II, Table XXIII.

Each result is an average of five determinations ± Standard Deviation of the Mean.

In the present work, it was found that there was no polarographic wave for neomycin sulphate in the whole pH range. According to the literature (148), this antibiotic can be hydrolysed with mineral acid to furfural and an organic base. The furfural can be reduced like any other aldehyde. With 37% HCl the most suitable conditions for hydrolysis of neomycin sulphate were found to be as follows:

(i) volume of 37% HCl = 10 ml. for concentrations up to 2.50 mg. of the antibiotic

(ii) time of hydrolysis - 15 minutes

(iii) temperature of hydrolysis = 95 ± 2°C.

The hydrolysed mixture gave two waves throughout the pH range. The second wave was flat throughout and not fully developed at certain values of pH. In phosphate buffer, pH 7.20 was found to be most suitable for measurement of the first wave.

The first wave was recorded at -0.99 v. and the second wave if it were fully developed would have been at approximately -1.48 v. The results are shown in Table LXIII and Figure 20. The results of the microbiological comparison are shown in Table LXIV and Figure 21.

V. Erythromycins

(i) Erythromycin

Erythromycin, a fine white crystalline compound, could be analysed polarographically. The material was not completely soluble

Table LXIII

Data on the Diffusion Currents of the First Wave of Neomycin Sulphate in Phosphate Buffer at pH 7.20 ± 0.05. $m^{2/3}t^{1/6}$ = 1.69 mg. $^{2/3}$ Sec. $^{-1/2}$

Concentration mg./10 ml.	Diffusion Current 100 units = 3 µa.	Average ± S.D.M.
0.25	1.67 1.67 1.83 1.67 1.67	1.7 ± 0.1
0.50	2.67 2.83 2.83 2.67 2.83	2.7 ± 0.1
0.75	4.12 4.00 4.00 3.87 4.00	4.0 ± 0.1
1.00	5.25 5.50 5.25 5.00 5.25	5.2 ± 0.2
1.25	6.50 6.75 6.00 6.50 6.50	6.5 ± 0.2
1.50	7.75 7.25 7.50 7.50 7.50	7.5 ± 0.2
1.75	8.75 9.00 8.50 8.50 8.75	8.7 ± 0.2
2.00	10.00 10.25 9.75 9.50 9.75	9.8 ± 0.3

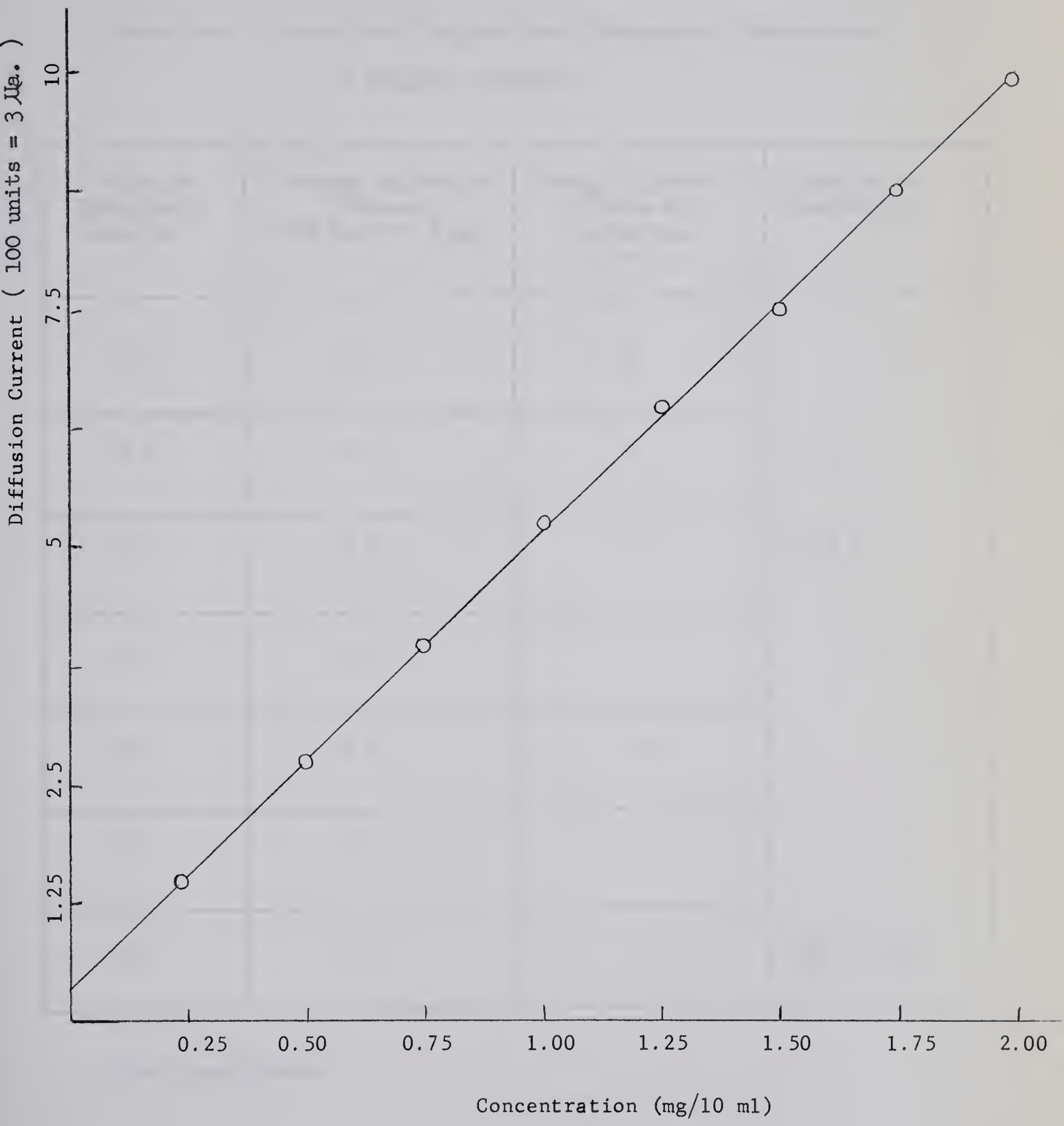


Figure 20. The graph of Neomycin Sulphate standard.

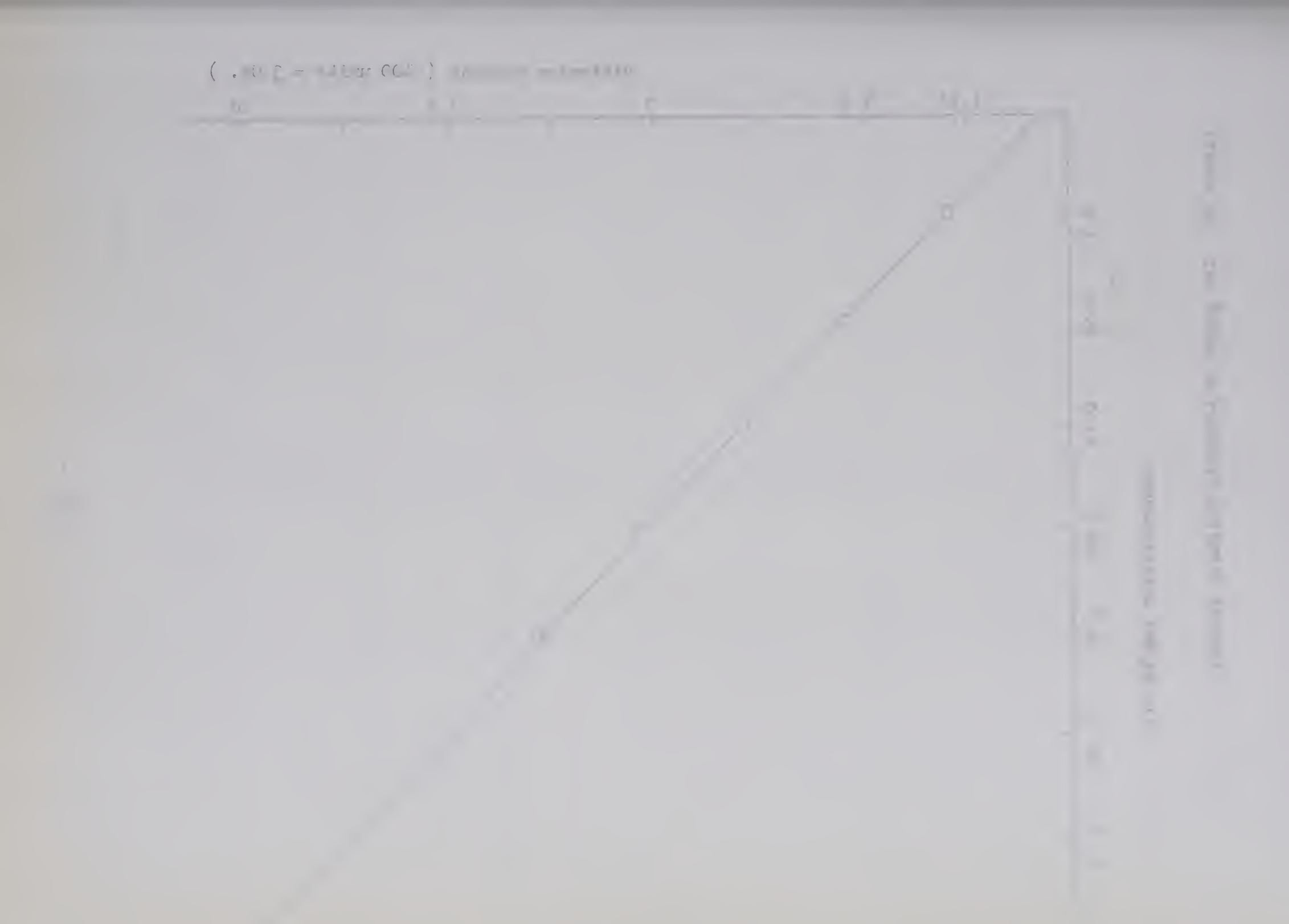


Table LXIV

Comparison of the Microbiological and Polarographic Measurement of Neomycin Sulphate

Time of Hydrolysis minutes.	Average Diffusion Current 100 Units = 3 μa.	Average Diameter of zone of inhibition mm.	Correlation Coefficient r
1.0	0.7	18	
2.5	1.5	16	
5.0	2.8	13	1.47 *
7.5	3.2	4	
10	3.6	0.5	
15	3.8	0	
20	3.8	0	95% = 0.811 99% = 0.917

* Not significant.

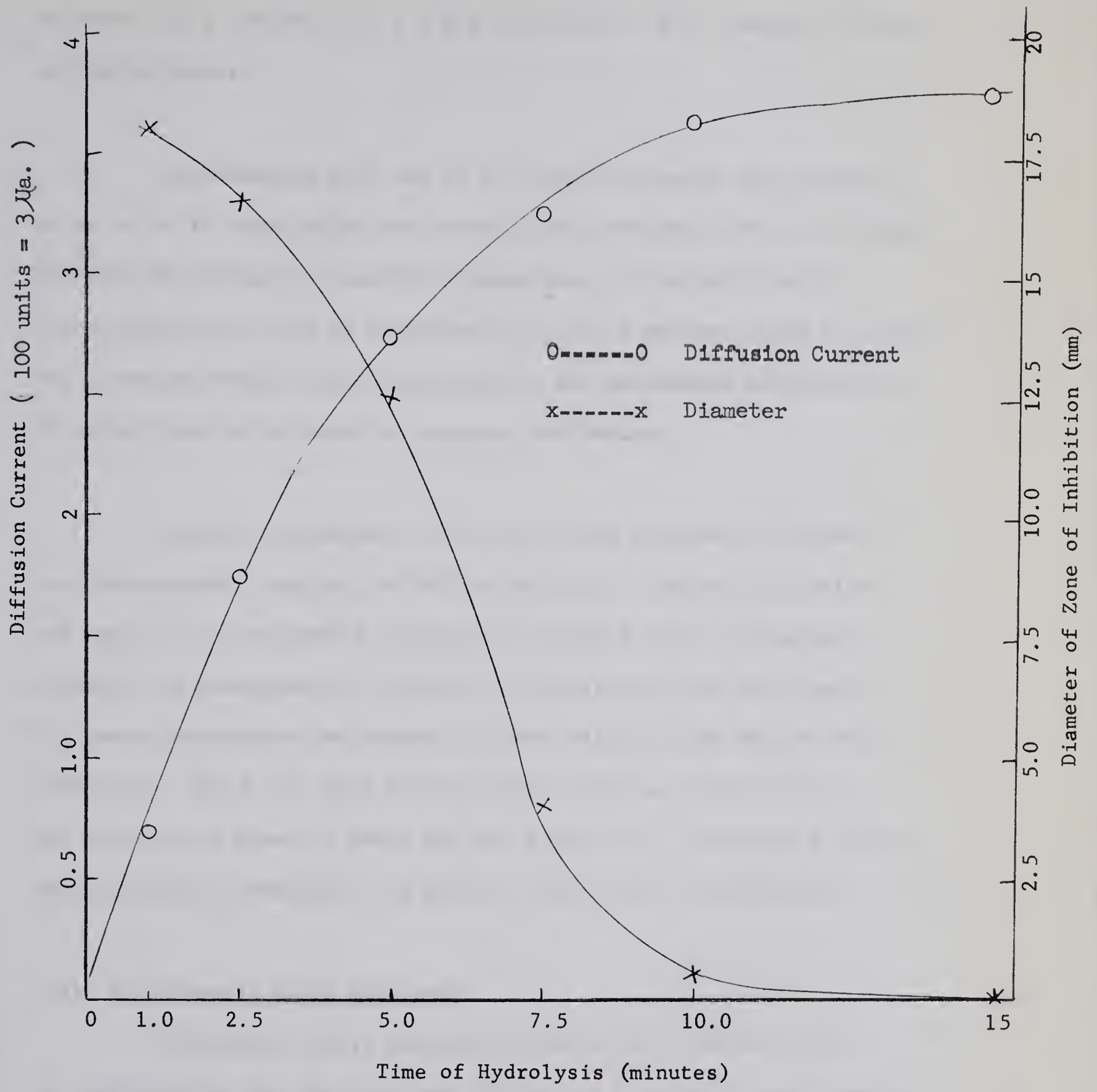
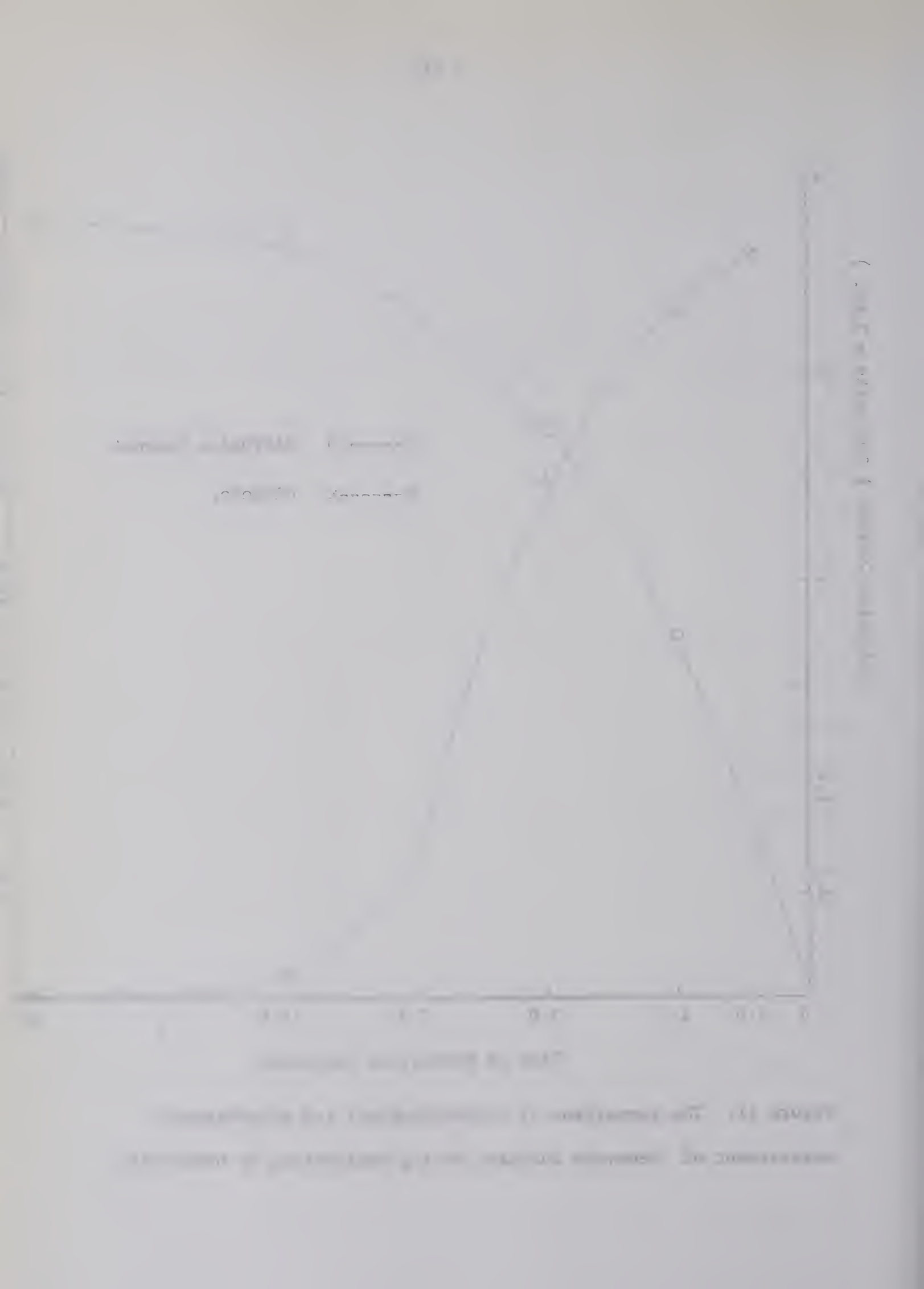


Figure 21. The comparison of microbiological and polarographic measurement of Neomycin Sulphate during degradation by hydrolysis.



in water and a few drops of 3 M HCl was added to give complete solution of the antibiotic.

Erythromycin gave one or two reduction waves from the acid pH up to pH 10 after which the waves became very small and at extremely alkaline pH the waves completely disappeared. Starting from the concentration of 0.125 mg erythromycin/10 ml, a maximum began to appear, and increased rapidly with concentration and increasing concentration of gelatin had to be added to suppress the maximum.

Borate or phosphate buffer was found unsuitable for use in the polarographic analysis of this antibiotic. Potassium chloride was used as the supporting electrolyte and pH 6.50 was found most suitable for measurement of diffusion current from the first wave. The second wave which was present at some values of pH was not well developed. The E 1/2 value for the first wave was -1.60 ± 0.01 v. The results are shown in Table LXV and Figure 22. The results of the microbiological comparison are shown in Table LXVI and Figure 23.

(ii) Erythromycin Ethyl Succinate

Erthromycin ethyl succinate behaved in a similar manner to erythromycin, but the wave was obtained at a slightly less negative potential. It gave a single wave in the pH range from low pH to pH 10.50 beyond which the wave was very small and then at 11.50 it completely disappeared.

Table LXV

Data on the Diffusion Currents of the First Wave of Erythromycin at pH

$$6.50 \pm 0.05.\ m^{2/3}\ t^{1/6} = 1.62\ mg.^{2/3}\ Sec.^{-1/2}$$

Concentration mg./10 ml.	Diffusion Current 100 units = 3 Ua.	Average ± S.D.M.
0.125	1.33 1.50 1.33 1.33 1.33	1.4 ± 0.0
0.250	2.83 2.67 2.83 2.67 2.67	2.7 ± 0.1
0.375	4.00 4.00 4.00 4.00 3.87	4.0 ± 0.1
0.500	5.25 5.00 5.00 5.00 5.00	5.0 ± 0.1
0.75	7.75 8.00 7.75 7.75 7.75	7.8 ± 0.1
1.00	10.50 10.50 10.25 10.50 10.75	10.5 ± 0.2
1.25	12.75 13.00 13.00 13.25 13.00	13.0 ± 0.3
1.50	15.50 16.00 15.25 15.75 15.75	15.6 ± 0.3

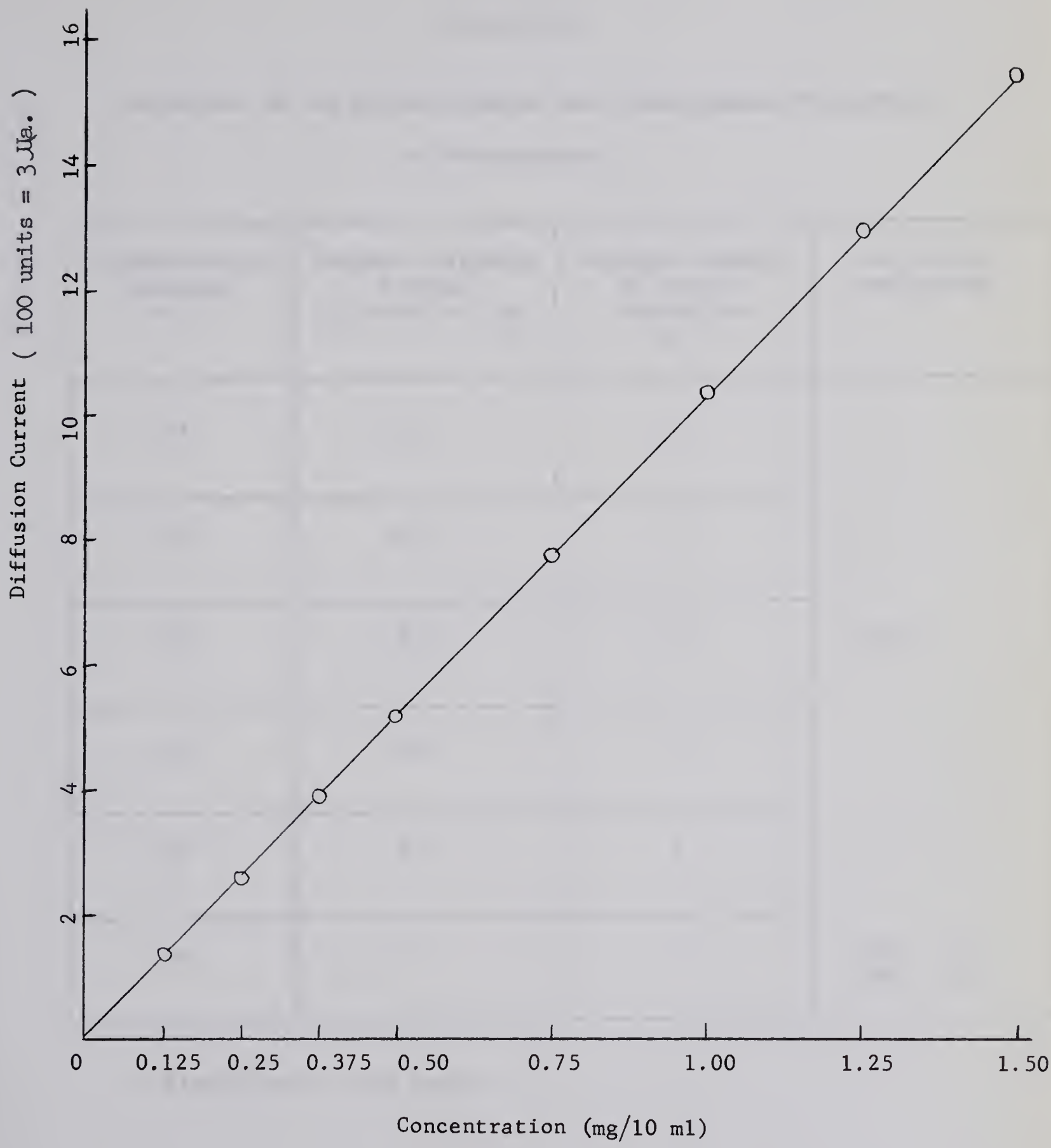


Figure 22. The graph of Erythromycin standard.

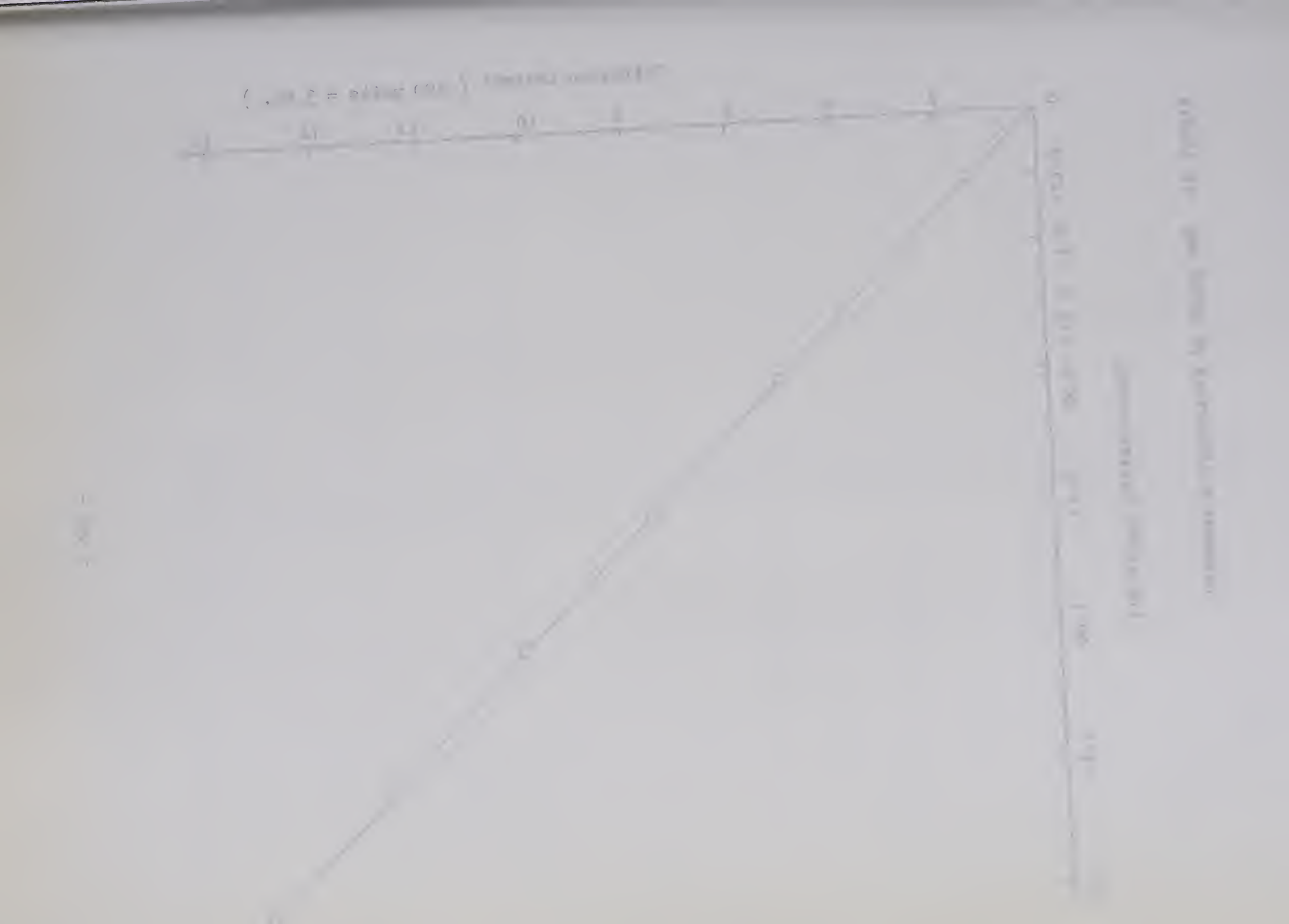


Table LXVI

Comparison of the Microbiological and Polarographic Measurement of Erythromycin.

Temperature of Heating 0^{o} C	Average Diffusion Current 100 Units = 3 µa.	Average Diameter of zone of inhibition mm.	Correlation coefficient r
100	21.0	16	
110	21.2	16	
120	18.0	12	0.99*
125	12.5	8	
130	8.0	5	
140	0	0	95% = 0.95 99% = 0.99

* Significant at 99% level.

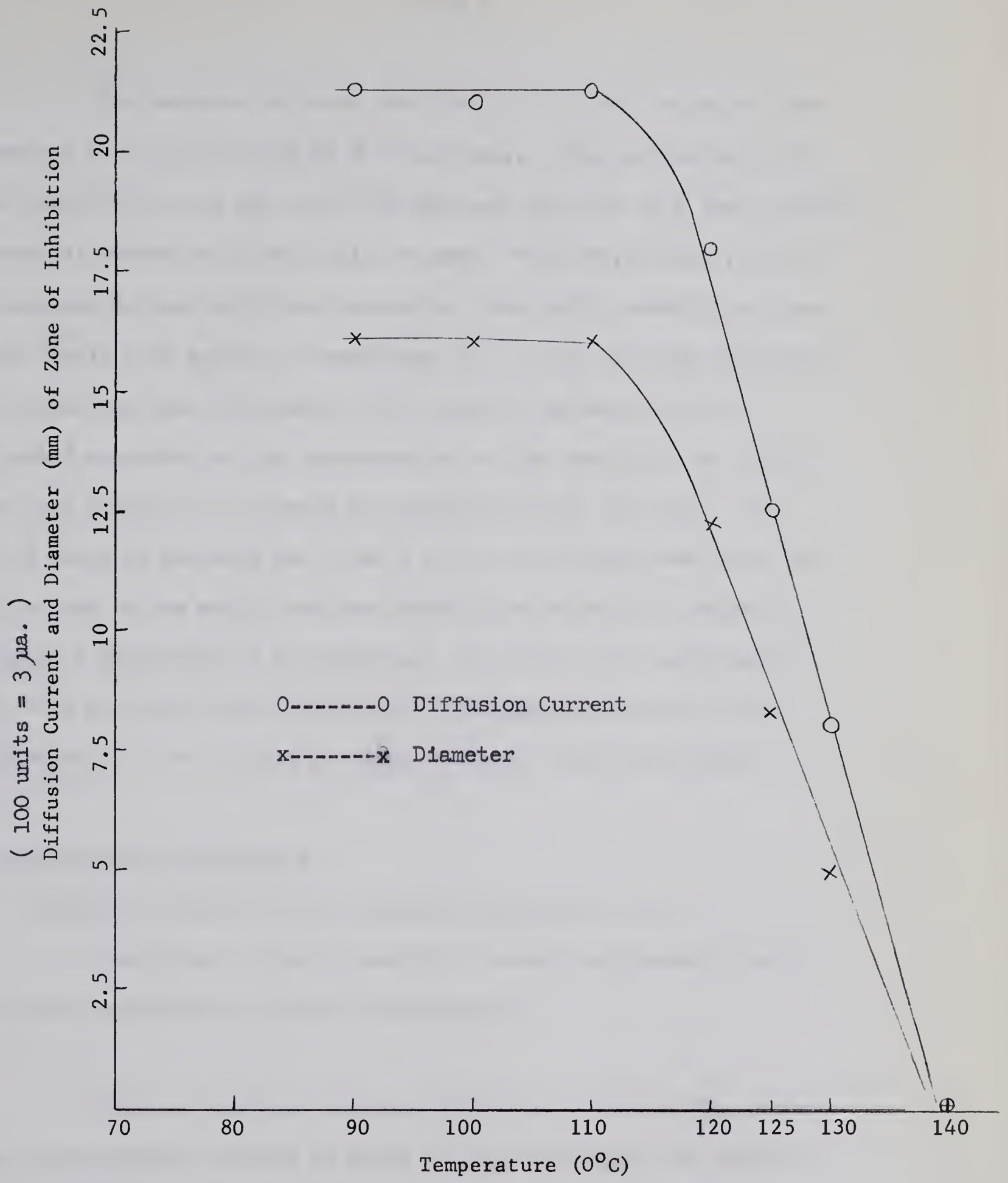


Figure 23. The comparison of microbiological and polarographic measurement of Erythromycin during degradation.

The workable pH range was from 5.00 - 8.00; below pH 5 the wave was too big and above pH 8 it was small. The most suitable pH was found to be 6.50 and since the wave was obtained at a less negative potential phosphate buffer could be used. The wave, however, had a pronounced maximum which was removed at first quite readily and then more slowly with gelatin or methylene blue. With too much suppressor the peak was lost. Therefore, the amount of gelatin had to be adjusted according to the concentration of the antibiotic so that it was just sufficient to remove the maximum and not the peak. The E 1/2 value of the wave was -1.48 ± 0.01 v. This wave was found to be not due to the ethyl succinate part of the molecule as shown by running a polarogram of this compound. It could not be ascertained why this antibiotic gave the wave at less negative potential than erythromycin. The results are shown in Table LXVII and Figure 24.

(ii)a. Pharmaceutical Preparations

1. Erythrocin Chewable Tablet (Abbott Laboratories Ltd.)

Each green tablet is stated to contain erythromycin ethyl succinate equivalent to 200 mg. erythromycin.

Five tablets were powdered finely in a mortar. The powder was not completely soluble in water but the solubility was somewhat improved with 3 M HCl or alcohol. A combination of a few drops of 3 M HCl and 10% alcohol was used for best results leaving only some white pulpy material undissolved. The standard solution was prepared in the same manner. The analysis was carried out in phosphate buffer

Table LXVII

Data on the Diffusion Currents of the Single Wave of Erythromycin Ethyl Succinate in Phosphate Buffer at pH 6.50 ± 0.05.

$$m^{2/3} t^{1/6} = 1.63 \text{ mg.}^{2/3} \text{ Sec.}^{-1/2}$$

Concentration mg./10 ml.	Diffusion Current 100 units = 3 μa.	Average ± S.D.M.
0.025	4.50 4.50 4.50 4.67 4.50	4.5 ± 0.1
0.050	7.75 7.75 8.00 7.75 7.50	7.8 ± 0.2
0.125	16.75 16.00 16.50 15.75 16.25	16.2 ± 0.4
0.250	33.00 31.50 32.75 33.25 31.75	32.4 ± 0.8
0.375	46.25 47.50 47.92 47.92 45.42	47.0 ± 1.1
0.500	65.00 61.67 62.50 62.50 60.83	62.5 ± 1.6

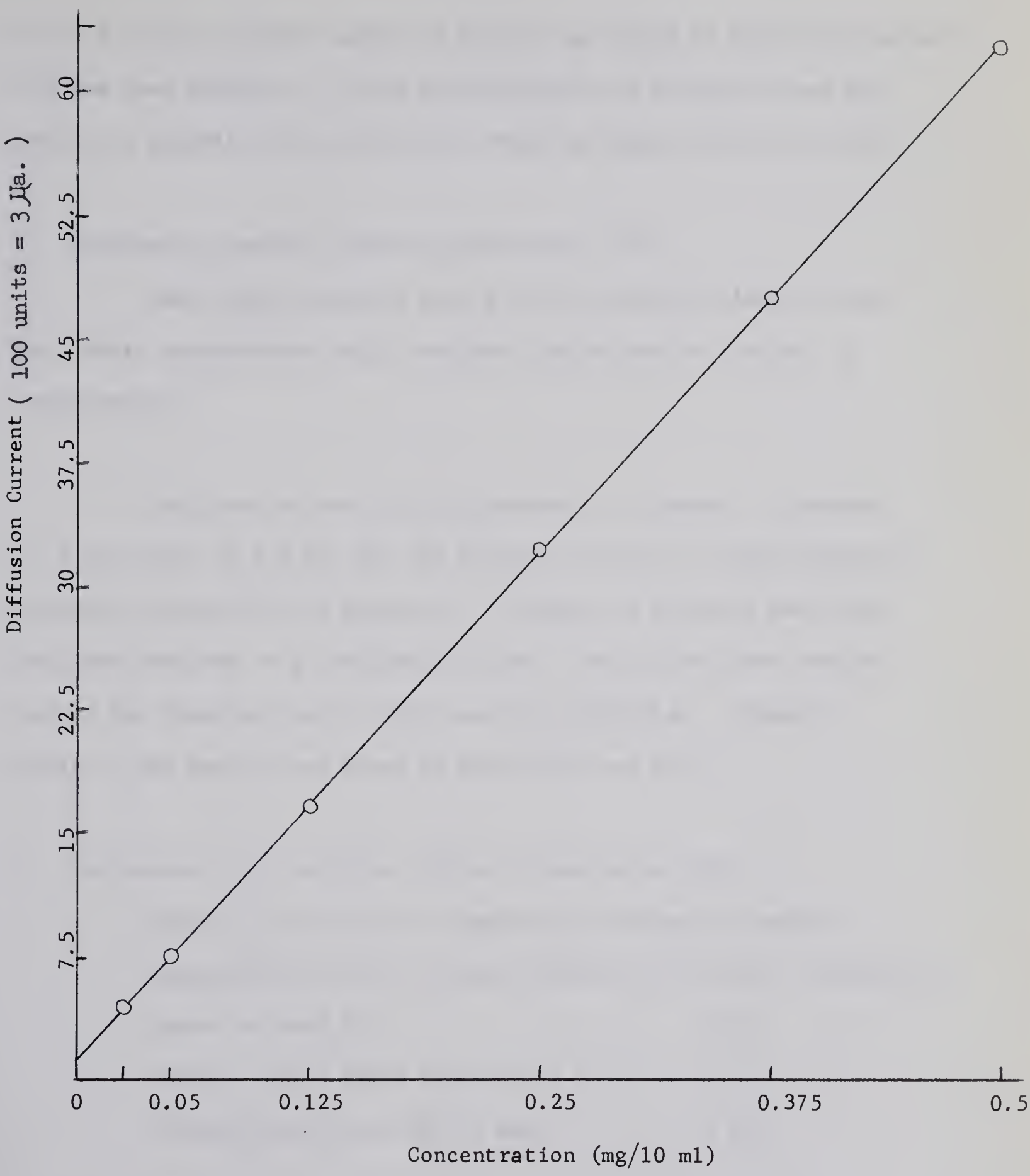


Figure 24. The graph of Erythromycin Ethyl Succinate standard.

at pH 6.50 and an equal amount of gelatin was added to both the standard and the test solution. As the concentration was changed so was the amount of gelatin. The results are shown in Tables LXVIII and LXIX.

2. Erythrocin Granules (Abbott Laboratories Ltd.)

Each vial containing rose pink buffered granules is stated to contain erythromycin ethyl succinate equivalent to 2.40 gm. of erythromycin.

The granules were finely powdered in a mortar. A mixture of a few drops of 3 M HCl and 10% alcohol was used to obtain the best possible solution of the granules. A variety of solvents was tried and none resulted in a complete solution. The analysis was carried out in the identical way of that used for Erythrocin Chewable Tablet. The results are shown in Tables LXX and LXXI.

3. Erythrocin I. M. Solution (Abbott Laboratories Ltd.)

Each ml. of the two ml. ampoules is stated to contain

Erythromycin ethyl succinate equivalent to	50 mg. erythromycin
Benzyl alcohol N.F.	0.9%
Butesin (Butyl amino benzoate, N.F.)	2.0%
Polyethylene glycol USP to make	1 ml.

The reduction wave of the preparation was obtained at a more negative potential than that of the pure erythromycin ethyl succinate,

Table LXVIII

Results on the Polarographic Determination of the Concentration of Erythromycin Ethyl Succinate in Erythrocin Chewable Tablet (Abbott - 200 mg./tablet)

Amount of the preparation mg./10 ml.	Erythromycin Ethyl Succinate found mg./10 ml.	Equivalent Erythromycin mg./10 ml.	Erythromycin/ tablet mg.	% Stated Concentration
0.25	0.088 ± 0.002	0.071 ± 0.001	210.1 ± 3.9	105.0 ± 1.9
0.50	0.175 ± 0.003	0.141 ± 0.003	209.6 ± 4.2	104.8 ± 2.1
0.75	0.262 ± 0.005	0.212 ± 0.004	210.0 ± 4.0	105.0 ± 2.0
1.00	0.350 ± 0.006	0.283 ± 0.005	209.8 ± 3.8	104.9 ± 1.9
1.50	0.526 ± 0.012	0.426 ± 0.009	210.4 ± 4.6	105.2 ± 2.3

Average = 105.0

Individual experimental results are presented in Appendix I, Table XXIV.

Each result is an average of five determinations ± Standard Deviation of the Mean.



Table LXIX

Results on the Polarographic Determination of the Recovery of Added Erythromycin Ethyl Succinate from Erythrocin Chewable Tablet.

Amount of the preparation mg./10 ml.	Erythromycin Ethyl Succinate				
	Amount added mg.	Total present mg./10 ml.	Total found mg./10 ml.	Amount recovered mg.	% Recovery
0.50 mg. ≡ 0.142 mg. Erythromycin ≡ 0.175 mg. Erythromycin Ethyl Succinate	0.10	0.275	0.275 ± 0.001	0.101 ± 0.001	101.0 ± 1.1
	0.20	0.375	0.375 ± 0.005	0.200 ± 0.005	100.0 ± 2.4
	0.30	0.475	0.475 ± 0.008	0.300 ± 0.008	100.0 ± 2.5
	0.35	0.525	0.525 ± 0.009	0.350 ± 0.009	100.0 ± 2.5
	0.40	0.575	0.574 ± 0.010	0.399 ± 0.010	99.8 ± 2.6

Average = 100.1 ± 0.5

Individual experimental results are presented in Appendix II, Table XXIV.

Each result is an average of five determinations ± Standard Deviation of the Mean.

Table LXX

Results on the Polarographic Determination of the Concentration of Erythromycin Ethyl Succinate in Erythrocin Granules (Abbott - 2.4 gm./vial)

Amount of the preparation mg./10 ml.	Erythromycin Ethyl Succinate found mg./10 ml.	Erythromycin Ethyl Succinate/vial gm.	% Stated Concentration
0.50	0.059 ± 0.001	2.518 ± 0.038	104.9 ± 1.4
1.00	0.118 ± 0.002	2.510 ± 0.053	104.6 ± 2.2
1.50	0.177 ± 0.003	2.502 ± 0.041	104.2 ± 1.7
2.50	0.295 ± 0.005	2.505 ± 0.043	104.4 ± 1.8
3.50	0.412 ± 0.008	2.494 ± 0.047	103.9 ± 2.0

Average = 104.4

Individual experimental results are presented in Appendix I, Table XXV.

Each result is an average of five determinations ± Standard Deviation of the Mean.

Table LXXI

Results on the Polarographic Determination of the Recovery of Added Erythromycin Ethyl Succinate from Erythrocin Granules.

Amount of the preparation mg/10 ml.	Erythromycin Ethyl Succinate				
	Amount added mg.	Total present mg./10 ml.	Total found mg./10 ml.	Amount recovered mg.	% Recovery
1.0 mg. = 0.118 mg. Erythromycin Ethyl Succinate	0.10	0.218	0.218 ± 0.002	0.100 ± 0.002	100.0 ± 2.5
	0.15	0.268	0.267 ± 0.003	0.149 ± 0.003	99.1 ± 1.9
	0.20	0.318	0.319 ± 0.004	0.201 ± 0.004	100.3 ± 2.1
	0.25	0.368	0.368 ± 0.005	0.249 ± 0.005	99.8 ± 2.0
	0.30	0.418	0.418 ± 0.007	0.300 ± 0.007	100.0 ± 2.3

Average = 99.8

Individual experimental results are presented in Appendix II, Table XXV.

Each result is an average of five determinations ± Standard Deviation of the Mean.

although waves in the two cases were similar in shape. This shift was found to be due to the presence of polyethylene glycol and occurred gradually with increased concentration of the solvent until a concentration was obtained beyond which there was no further change in the E 1/2 and diffusion current. Polyethylene glycol also acted as a partial suppressor for the maximum present in the wave of the antibiotic.

Benzyl alcohol in concentration much higher than the proportion in which it is present in the preparation, produced a very small wave from -1.34 to -1.44 v, but the proportion in which it appears in the drug had no effect. The other component was not readily available and so its effect could not be ascertained. But since the wave of the preparation was found to be identical with that of the standard containing the same proportion of polyethylene glycol, it should not have any effect.

When polyethylene glycol was added during preparation of the standard in the same proportion as is present in the pharmaceutical preparation, analysis of this drug was feasible in the usual buffer and at the same pH and gelatin in appropriate amounts to suppress the maximum. The results are shown in Tables LXXII and LXXIII.

Table LXXII

Results on the Polarographic Determination of the Concentration of Erythromycin in Erythrocin I.M. Solution (Abbott - 100 mg./2 ml. ampoule)

Amount of the preparation ml./10 ml.	Erythromycin Ethyl Succinate found mg./10 ml.	Equivalent Erythromycin mg./10 ml.	Erythromycin/2 ml. ampoule mg.	% Stated Concentration
0.002	0.124 ± 0.002	0.100 ± 0.002	100.2 ± 1.8	100.2 ± 1.8
0.004	0.248 ± 0.006	0.201 ± 0.005	100.6 ± 2.6	100.6 ± 2.6
0.006	0.371 ± 0.008	0.300 ± 0.006	100.1 ± 2.0	100.1 ± 2.0
0.008	0.497 ± 0.009	0.402 ± 0.007	100.6 ± 1.8	100.6 ± 1.8
0.010	0.621 ± 0.013	0.502 ± 0.010	100.5 ± 2.1	100.5 ± 2.1

Average = 100.4

Individual experimental results are presented in Appendix I, Table XXVI.

Each result is an average of five determinations ± Standard Deviation of the Mean.

Table LXXIII

Results on the Polarographic Determination of the Recovery of Added Erythromycin Ethyl Succinate from Erythrocin I.M. Solution.

Amount of the preparation ml./10 ml.	Erythromycin Ethyl Succinate				
	Amount added mg.	Total present mg./10 ml.	Total found mg./10 ml.	Amount recovered mg.	% Recovery
0.005 ml. ≡ 0.251 mg. Erythromycin ≡ 0.310 mg. Erythromycin Ethyl Succinate	0.05	0.360	0.360 ± 0.001	0.050 ± 0.001	100.0 ± 2.2
	0.10	0.410	0.410 ± 0.003	0.100 ± 0.003	100.0 ± 2.6
	0.15	0.460	0.460 ± 0.003	0.150 ± 0.003	100.0 ± 2.1
	0.20	0.510	0.510 ± 0.005	0.200 ± 0.005	100.0 ± 2.4
	0.25	0.560	0.561 ± 0.005	0.251 ± 0.005	100.4 ± 1.9

Average = 100.2 ± 0.2

Individual experimental results are presented in Appendix II, Table XXVI.

Each result is an average of five determinations ± Standard Deviation of the Mean.

VI. Penicillins

(i) Procaine Penicillin G

The polarographic determination of penicillin has been investigated (149). Procaine penicillin can be analysed for both the components. The hydrolysis of penicillin yields penillo-aldehyde and penicillamine (150) both of which most likely undergo nitrosation. Under these conditions procaine is not nitrosated. On the other hand, if hydrolysis is not carried out, nitrosation occurs with procaine only. In both cases the nitrosated compounds are reducible polarographically.

In the determination of the penicillin part, some variations of the conditions previously used (149) were attempted to improve the shape of the waves. The optimum conditions were found to be as follows:

(i)	volume of 37% HCl for hydrolysis	= 0.75 ml for up to 5.00 mg. of the antibiotic
(ii)	temperature of hydrolysis	= 65 ± 2°C
(iii)	time of hydrolysis	= 15 minutes
(iv)	vol. of 1 M potassium nitrite for nitrosation	= 3 ml.

Polarography was carried out throughout the pH range and a pH of 11.35 was found most suitable. The previous workers had used a pH of 7.0 (149). There were two waves both of which could be measured quantitatively but the first wave was better for this purpose. The half-wave potentials were -0.65 and -0.98 v. respectively for the

first and second wave. At concentrations as high as 10.00 mg/10 ml another small wave appeared at -1.31 v. The results are shown in Table LXXIV and Figure 25.

Procaine penicillin G could be measured directly by determining the single wave at pH 8.00 which was found to be due to the procaine moiety present in this antibiotic. The antibiotic gave one or two reduction waves throughout the pH range and a definite single wave at pH 8.00. With potassium chloride as the base electrolyte, this wave was quite small, but if borate buffer was used the wave became appreciably larger. The wave appeared at the same potential as that of pure procaine. The E 1/2 was -1.70 ± 0.01 v. At concentrations below 0.20 mg of procaine penicillin G/10 ml, the peak was difficult to measure and a maximum appeared at concentrations higher than 2.50 mg of the antibiotic/10 ml. The results are shown in Table LXXV and Figure 26.

(ii) Potassium Penicillin G

Following the same nitrosation procedure as for procaine penicillin G, potassium penicillin G could be measured by polarography. Potassium penicillin G gave two good waves from pH 5.50 up to the extreme alkaline pH. The most suitable pH was found to be 10.50. The half-wave potentials for the two waves were -.98 ± 0.01 v. and -1.31 ± 0.02 v. The results are shown in Table LXXVI and Figure 27.

Table LXXIV

Date on the Diffusion Currents of the Two Waves of Procaine Penicillin G

at pH 11.35 ± 0.05. $m^{2/3} t^{1/6} = 1.61$ mg. $^{2/3}$ Sec. $^{-1/2}$

Concentration mg/10 ml.	First Wave		Second Wave	
	Diffusion Current 100 units = 3 µa.	Average ± S.D.M.	Diffusion Current 100 units = 3 µa.	Average ± S.D.M.
0.25	4.25 4.00 4.00 4.00 4.00	4.0 ± 0.1	10.25 10.50 10.00 10.25 10.25	10.2 ± 0.2
0.50	7.75 7.75 7.50 7.75 8.00	7.8 ± 0.2	19.00 19.50 18.50 19.25 19.00	19.0 ± 0.4
0.75	11.25 11.50 11.75 11.50 12.00	11.6 ± 0.3	27.75 27.00 28.00 26.75 27.50	27.4 ± 0.6
1.00	15.00 14.50 15.25 15.50 15.25	15.1 ± 0.4	36.75 38.00 36.25 37.75 37.25	37.2 ± 0.7
1.25	22.75 22.00 23.00 22.50 22.00	22.4 ± 0.4	53.33 54.58 52.92 53.33 55.00	53.8 ± 0.9
2.00	30.00 29.50 29.50 28.75 29.00	29.4 ± 0.6	70.25 71.00 71.75 73.00 72.50	71.7 ± 1.1

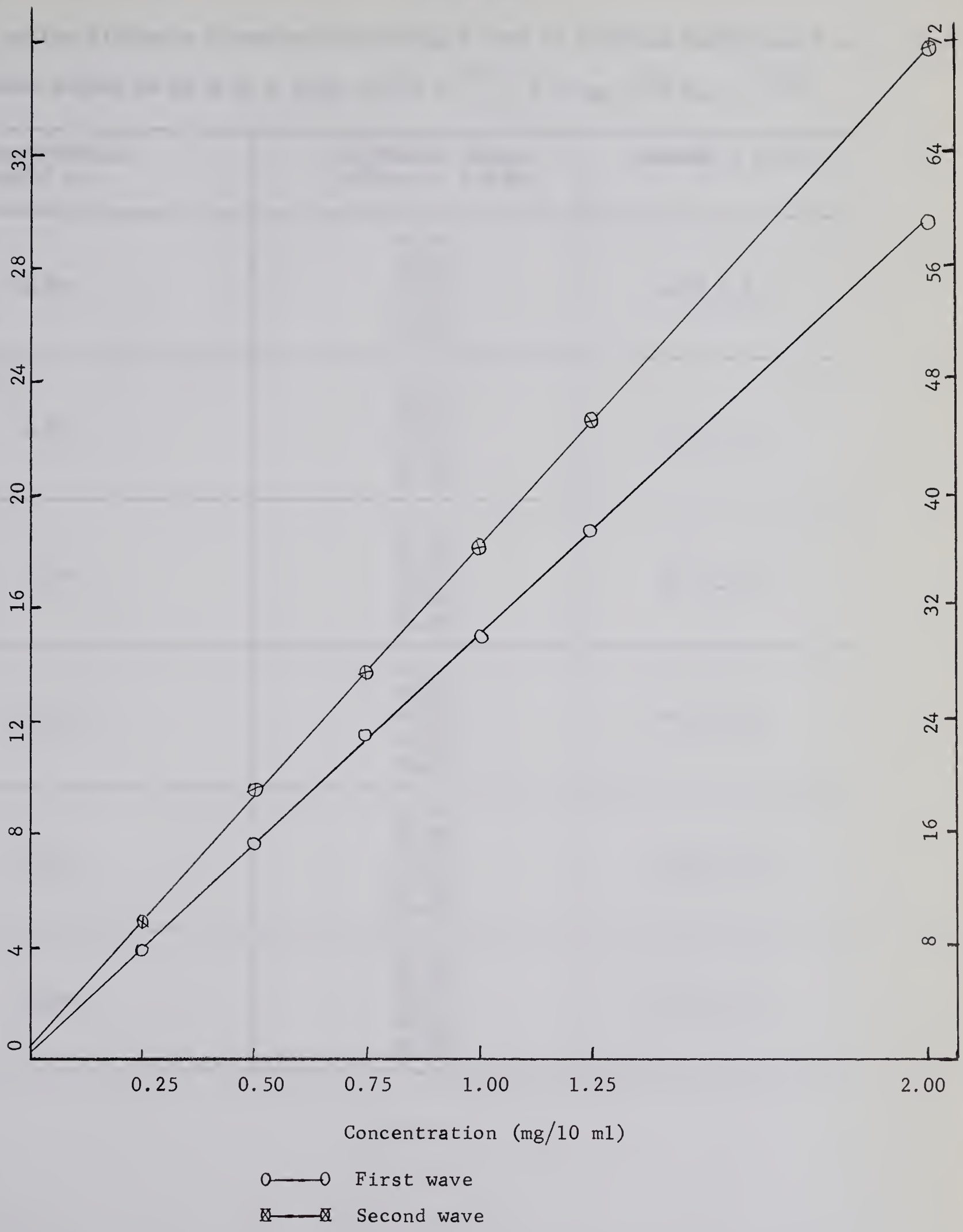


Figure 25. The graphs of Procaine Penicillin G standard (due to Penicillin moiety).

Table LXXV

Data on the Diffusion Currents of the Single Wave of Procaine Penicillin G in Borate Buffer at pH 8.00 ± 0.05. $m^{2/3} t^{1/6} = 1.62$ mg. $^{2/3}$ Sec. $^{-1/2}$.

Concentration mg./10 ml.	Diffusion Current 100 units = 3 Ua.	Average ± S.D.M.
0.250	20.75 20.25 20.50 19.75 20.00	20.2 ± 0.4
0.375	27.50 26.75 28.25 27.50 27.25	27.4 ± 0.5
0.500	35.75 37.25 35.25 37.00 36.25	36.3 ± 0.8
0.625	43.50 45.25 44.00 43.00 44.00	44.0 ± 0.8
0.750	51.67 52.08 53.75 52.92 50.83	52.2 ± 1.1
1.000	69.58 67.50 66.67 68.30 65.83	67.6 ± 1.4

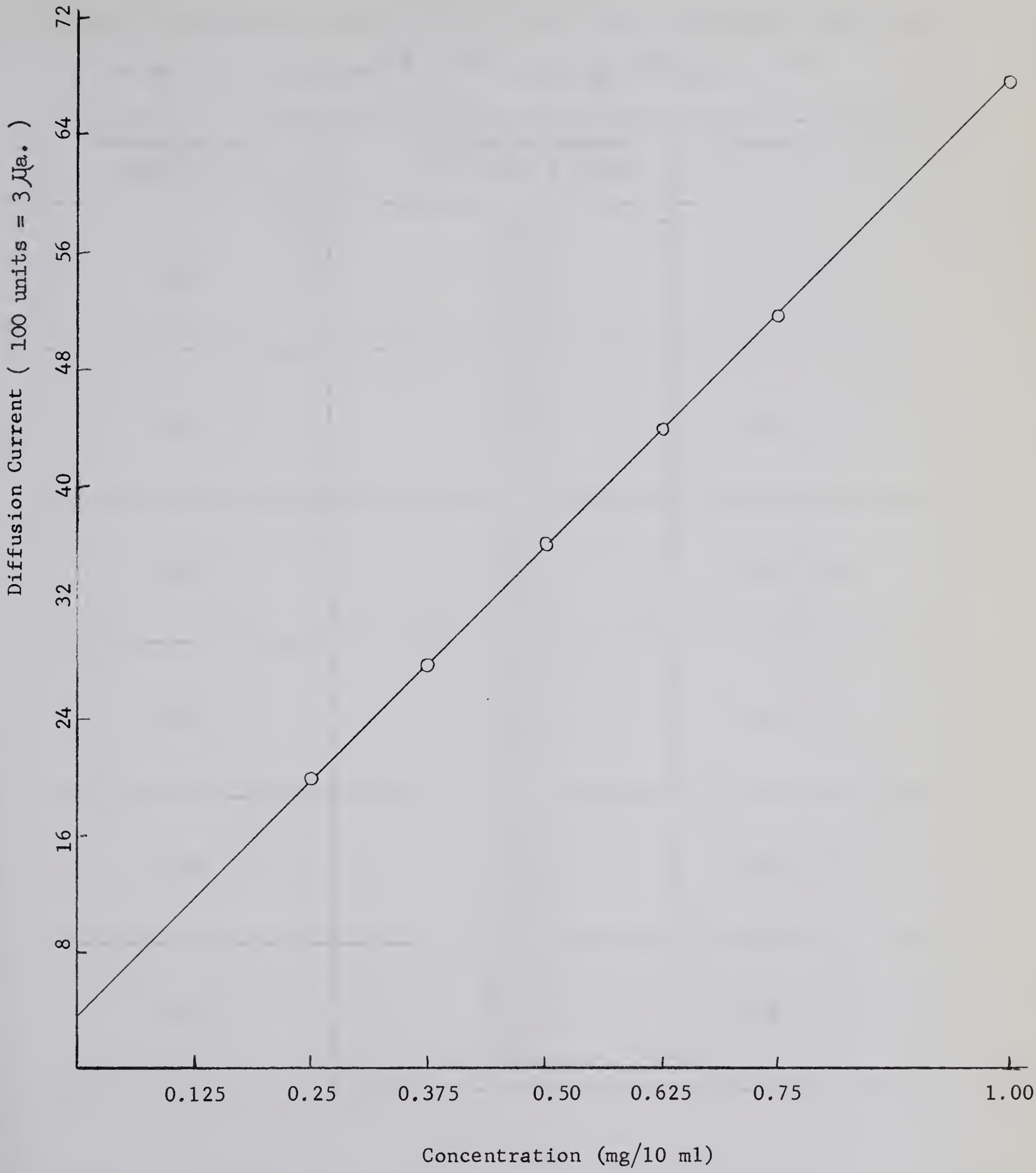


Figure 26. The graph of Procaine Penicillin G standard (due to Procain part).

Table LXXVI

Data on the Diffusion Currents of the First Wave of Potassium Penicillin G

at pH 10.50 ± 0.05. $m^{2/3}\ t^{1/6} = 1.69$ mg. $^{2/3}$ Sec. $^{-1/2}$

Concentration mg/10 ml.	Diffusion Current 100 units = 3 μa.	Average ± S.D.M.
0.25	3.50 3.38 3.50 3.50 3.50	3.5 ± 0.0
0.50	5.75 6.00 5.75 5.75 5.75	5.8 ± 0.1
0.75	7.75 8.00 8.00 8.25 8.00	8.0 ± 0.2
1.00	10.25 10.75 11.00 10.75 10.75	10.7 ± 0.2
1.50	15.50 16.00 15.25 15.00 15.50	15.5 ± 0.4
2.00	19.75 20.50 19.50 19.75 19.50	19.8 ± 0.4

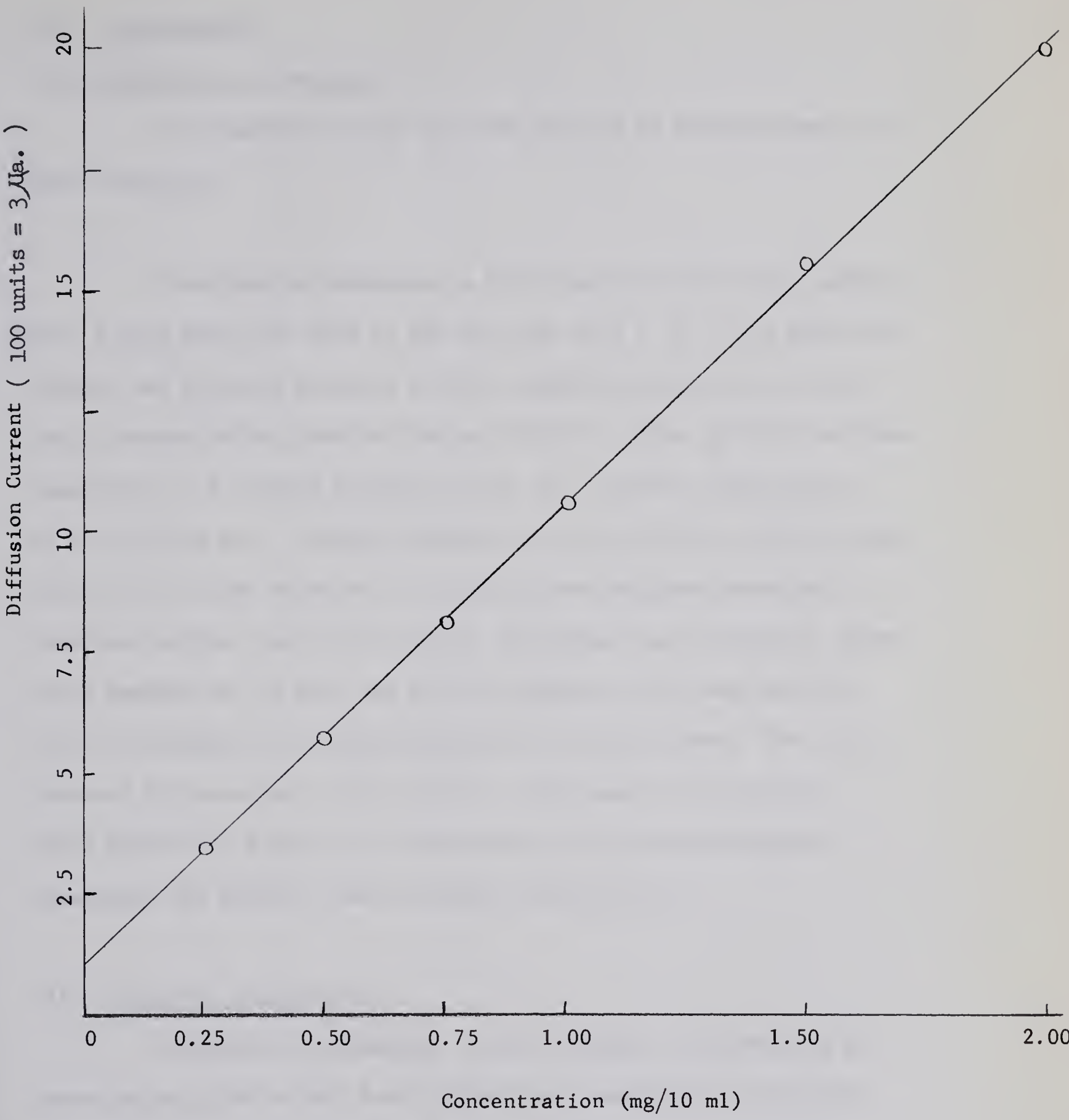


Figure 27. The graph of Potassium Penicillin G standard.

VII. Oleandomycins

(i) Oleandomycin Phosphate

Polarography has not yet been applied to the measurement of this antibiotic.

Oleandomycin phosphate, a white water soluble bulky powder, gave a good reduction wave in the pH range of 5 - 10. The wave was sharper and the peak occurred at less negative potential as the pH was increased in the case of this antibiotic. After pH 10.75 the wave disappeared. To obtain a sharp as well as a suitably large wave a pH of 7.75 was used. Either phosphate or borate buffer could be used. Since the wave was obtained at slightly less negative potential in phosphate buffer than borate buffer, the former was preferred. There was a maximum in the wave and 0.20 ml gelatin (0.1%) was used for the 10 ml sample of the test solution as the suppressor. The E 1/2 value of the wave was -1.75 ± 0.02 v. The results are shown in Table LXXVII and Figure 28. The results of the microbiological comparison are shown in Table LXXVIII and Figure 29.

(ii) Triacetyl Oleandomycin

Triacetyl oleandomycin, a white powder, was insoluble in water and at alkaline pH, but it dissolved completely in the acid pH obtained with a few drops of 3M HCl. The solution first prepared in acidic pH could be brought up to pH 8.00 but beyond this precipitation occurred. The antibiotic gave a single wave throughout the

Table LXXVII

Data on the Diffusion Current of the Single Wave of Oleandomycin Phosphate in Phosphate Buffer at pH 7.80 ± 0.05. $m^{2/3} t^{1/6} = 1.61$ mg. $^{2/3}$ Sec. $^{-1/2}$.

Concentration mg./10 ml.	Diffusion Current 100 units = 3 µa.	Average ± S.D.M.
0.125	1.67 1.67 1.78 1.67 1.78	1.7 ± 0.1
0.250	3.25 3.17 3.17 3.00 3.17	3.2 ± 0.1
0.500	6.25 6.00 6.50 6.25 6.25	6.2 ± 0.2
0.750	8.75 8.75 9.25 9.00 9.25	9.0 ± 0.2
1.000	12.50 12.00 12.25 12.50 11.75	12.2 ± 0.3
1.500	17.75 18.00 17.25 17.75 18.25	17.8 ± 0.4
2.000	23.75 24.00 22.75 23.25 23.50	23.4 ± 0.5
2.500	29.50 29.75 28.25 29.00 28.75	29.0 ± 0.6

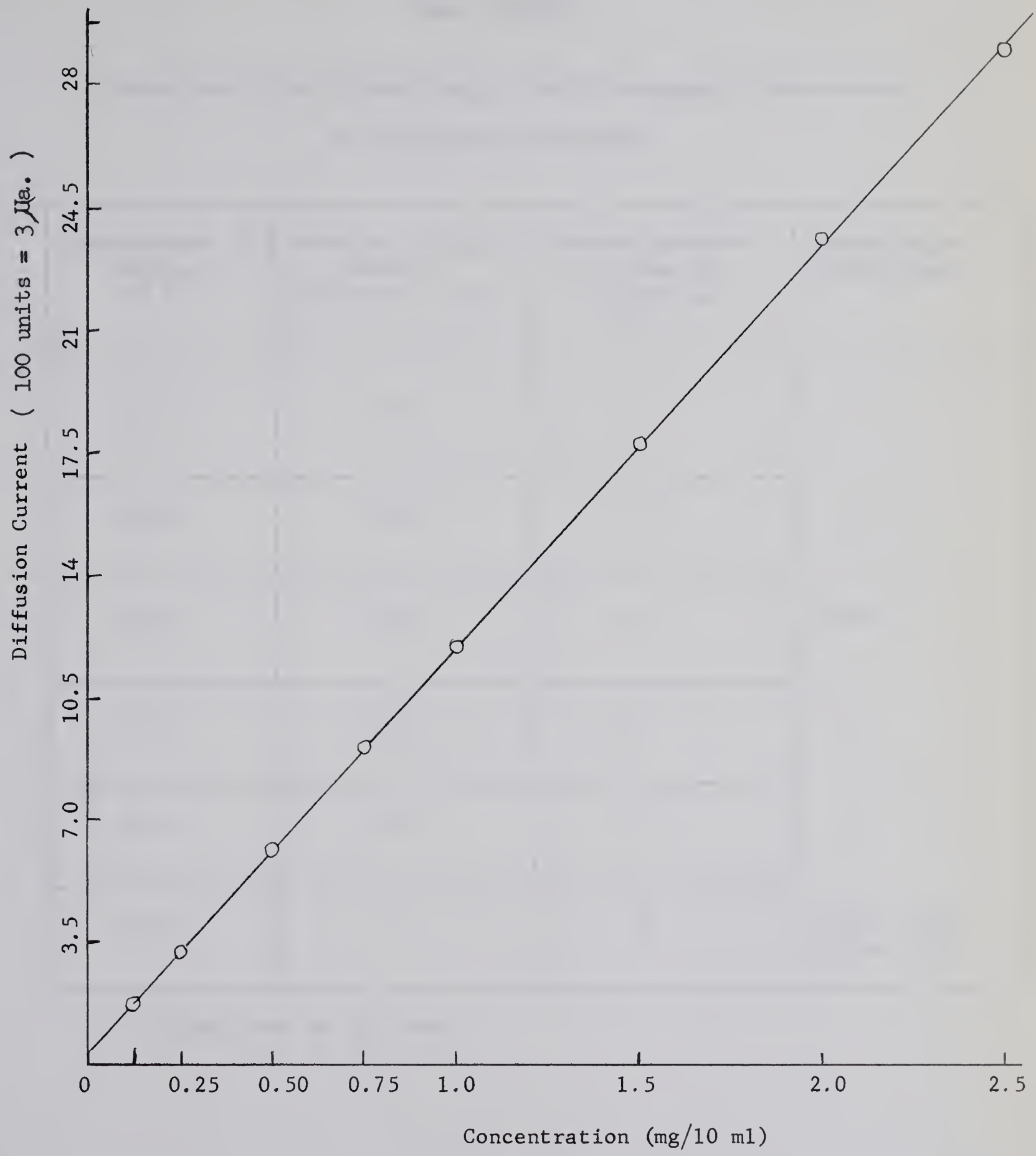


Figure 28. The graph of Oleandomycin Phosphate standard.

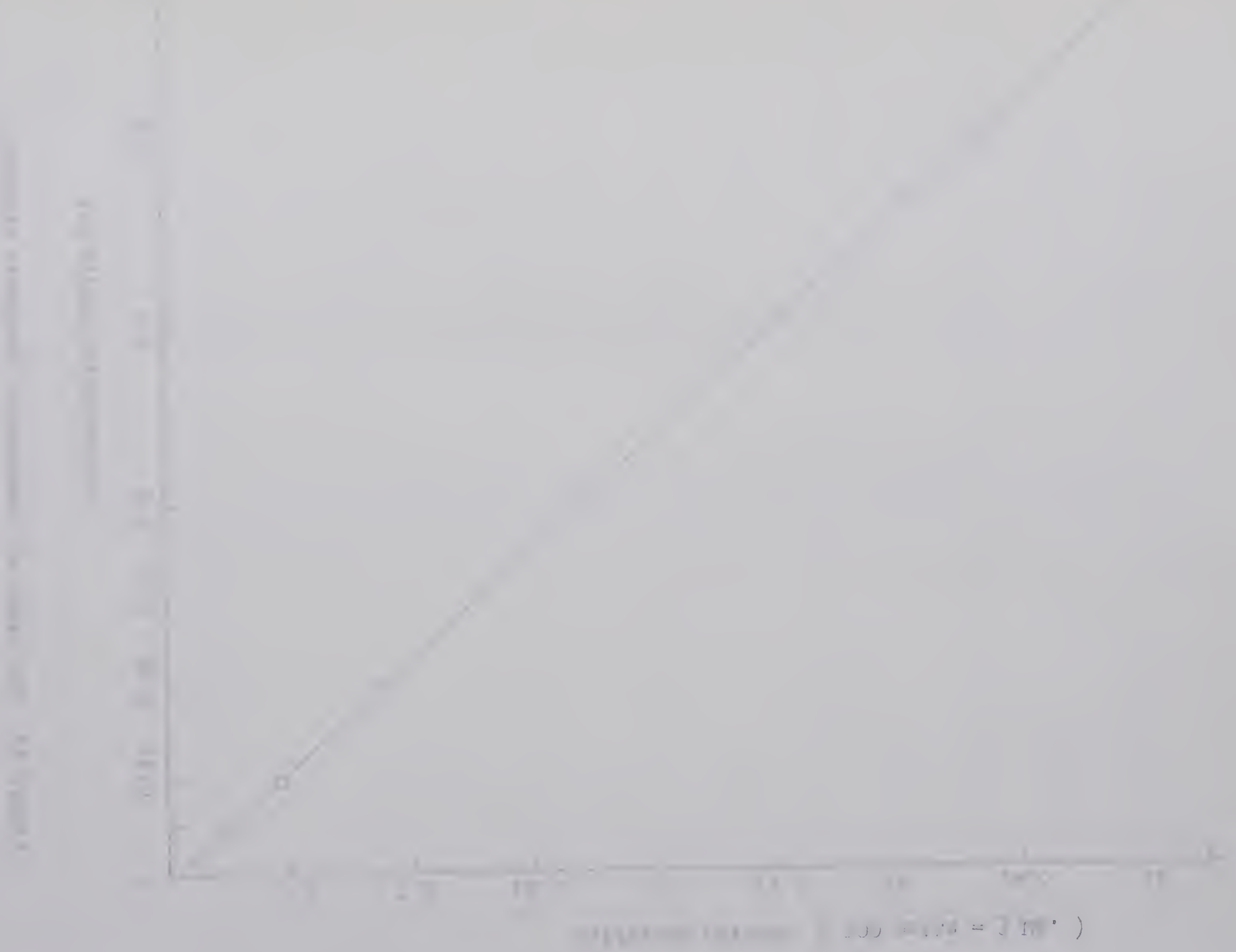


Table LXXVIII

Comparison of the Microbiological and Polarographic Measurement of Oleandomycin Phosphate.

Temperature of Heating 0^{o} C	Average Diffusion current 100 Units = 3 μa.	Average Diameter of zone of inhibition mm.	Correlation Coefficient r
100	23.2	16	
110	22.0	14	
120	18.0	12	0.99*
130	14.5	10	
135	8.0	6	
140	0	0	95% = 0.878 99% = 0.959

* Significant at 99% level.

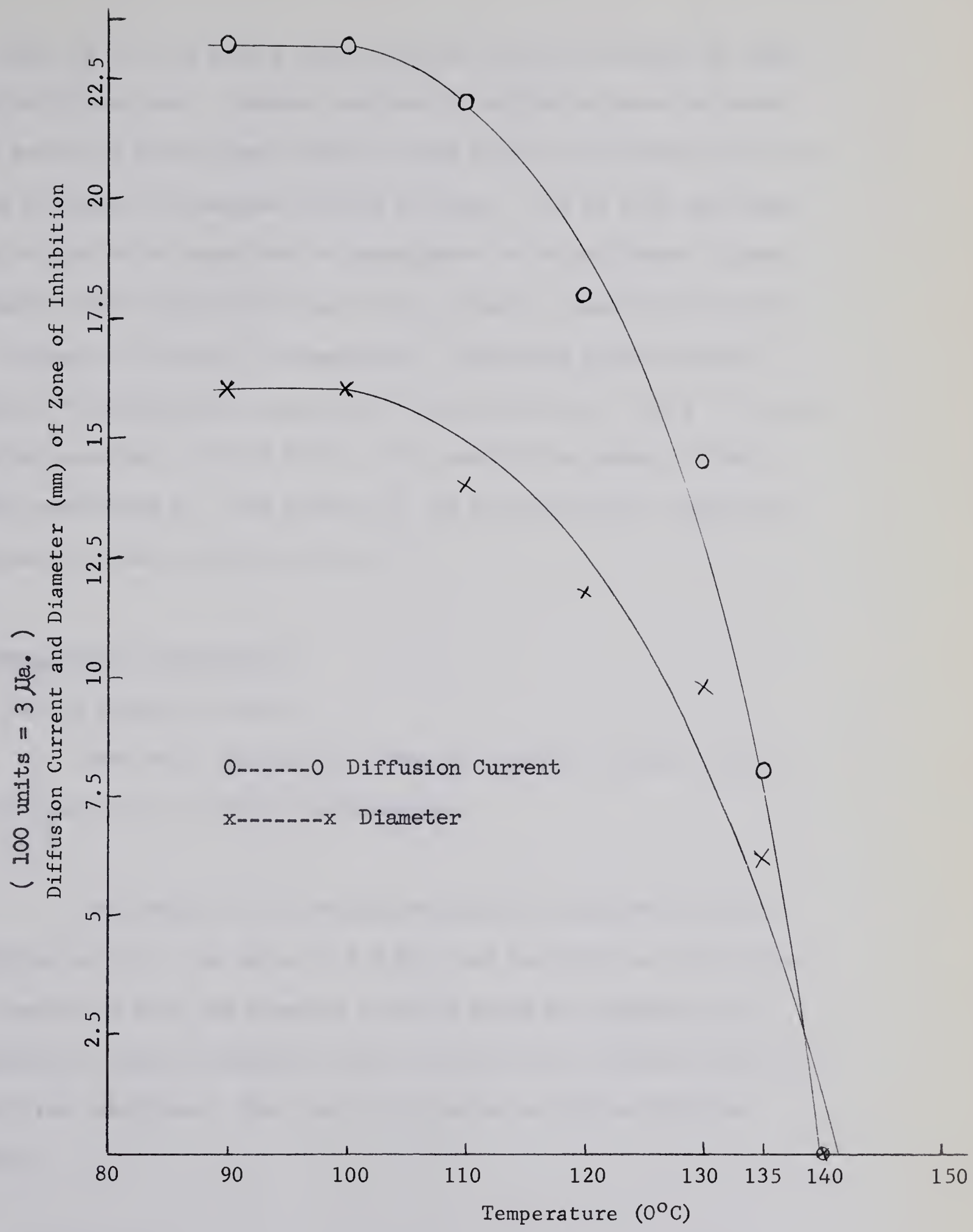


Figure 29. The comparison of microbiological and polarographic measurement of Oleandomycin Phosphate during degradation.

pH range up to 7.00 with a sharp decrease occurring before the peak was fully developed. Gelatin was used to stop this sharp decrease. The amount of gelatin was chosen in such a way that it was sufficient just to remove the maximum and not the peak. The pH 6.15 was found most suitable for quantitative measurement of the diffusion current. Acetate buffer could not be used since it gave a wave in the region of the wave of triacetyl oleandomycin. Phosphate buffer was most suited for polarographic analysis of the antibiotic. The E 1/2 value for the wave was - 1.61 ± 0.01 v. The results are shown in Table LXXIX and Figure 30. The results of the microbiological comparison are shown in Table LXXX and Figure 31.

(ii)a. Pharmaceutical Preparations

1. Olicin (Roerig, Canada)

Each white capsule is stated to contain triacetyl oleandomycin equivalent to 250 mg. oleandomycin.

The powder of the capsule was easily dissolved in water with the aid of a few drops of 3 M HCl, and analysis was carried out by comparison with the standard solution since the pharmaceutical preparation gave an identical wave to that of the standard under the specified conditions. The results are shown in Tables LXXXI and LXXXII.

VIII. Bacitracin

No report on the polarographic determination of bacitracin

Table LXXIX

Data on the Diffusion Currents of the Single Wave of Triacetyl Oleandomycin in Phosphate Buffer at pH 6.20 ± 0.05. $m^{2/3} t^{1/6} = 1.62$ mg. $^{2/3}$ Sec. $^{-1/2}$.

Concentration mg./10 ml.	Diffusion Current 100 units = 3 Ua.	Average ± S.D.M.
0.025	4.50 4.75 4.50 4.50 4.50	4.6 ± 0.1
0.050	10.25 10.50 10.50 10.75 10.50	10.5 ± 0.2
0.100	21.25 21.75 21.00 21.50 20.75	21.2 ± 0.4
0.150	31.00 32.50 31.75 31.50 31.25	31.6 ± 0.6
0.200	42.25 43.25 44.00 42.75 43.50	43.2 ± 0.6
0.300	65.42 66.67 64.17 65.00 64.58	65.2 ± 1.0
0.400	84.17 86.67 82.92 85.42 84.58	84.8 ± 1.4
0.500	105.00 101.67 106.67 105.83 105.00	104.8 ± 1.9

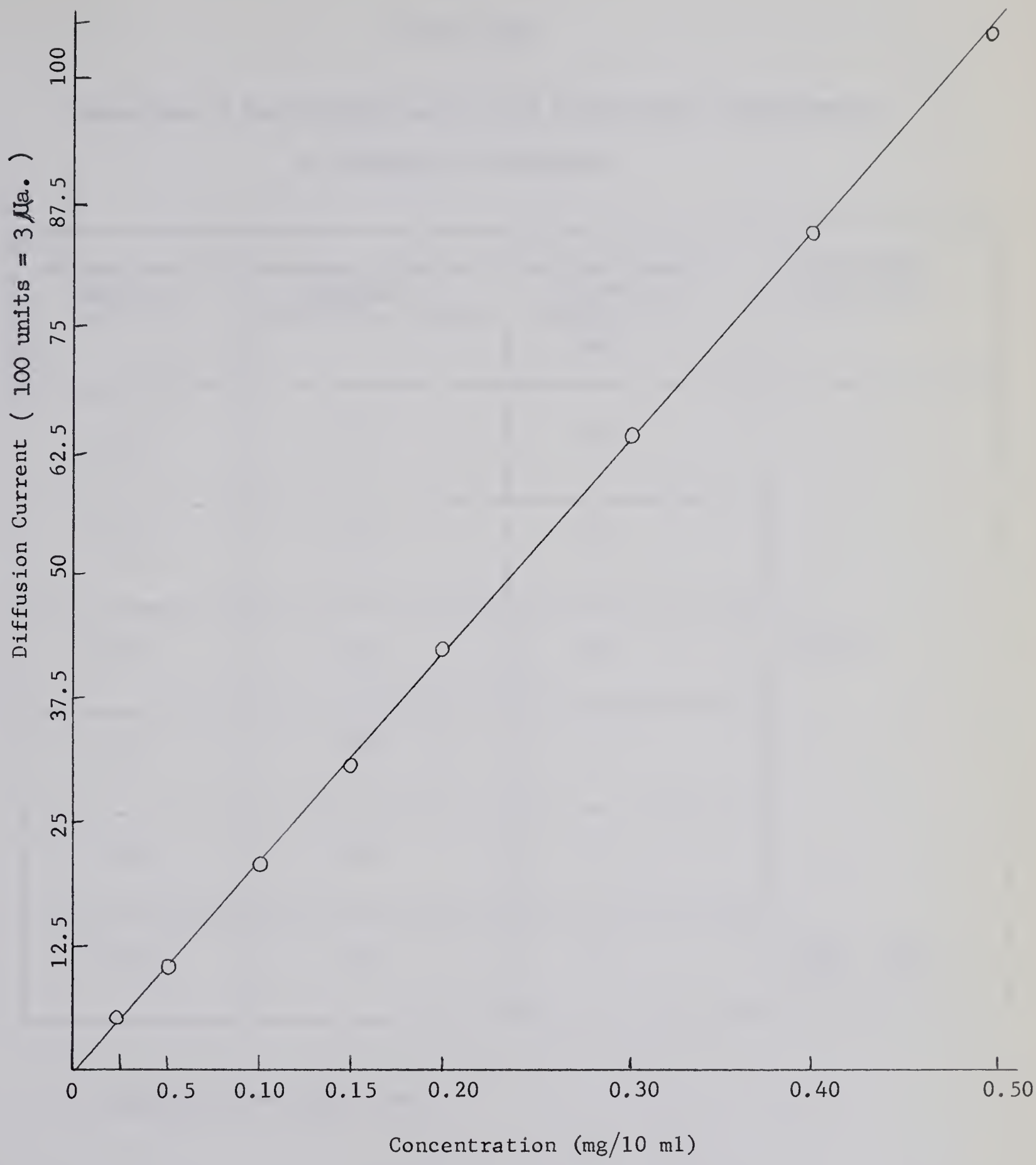


Figure 30. The graph of Triacetyl Oleandomycin standard.

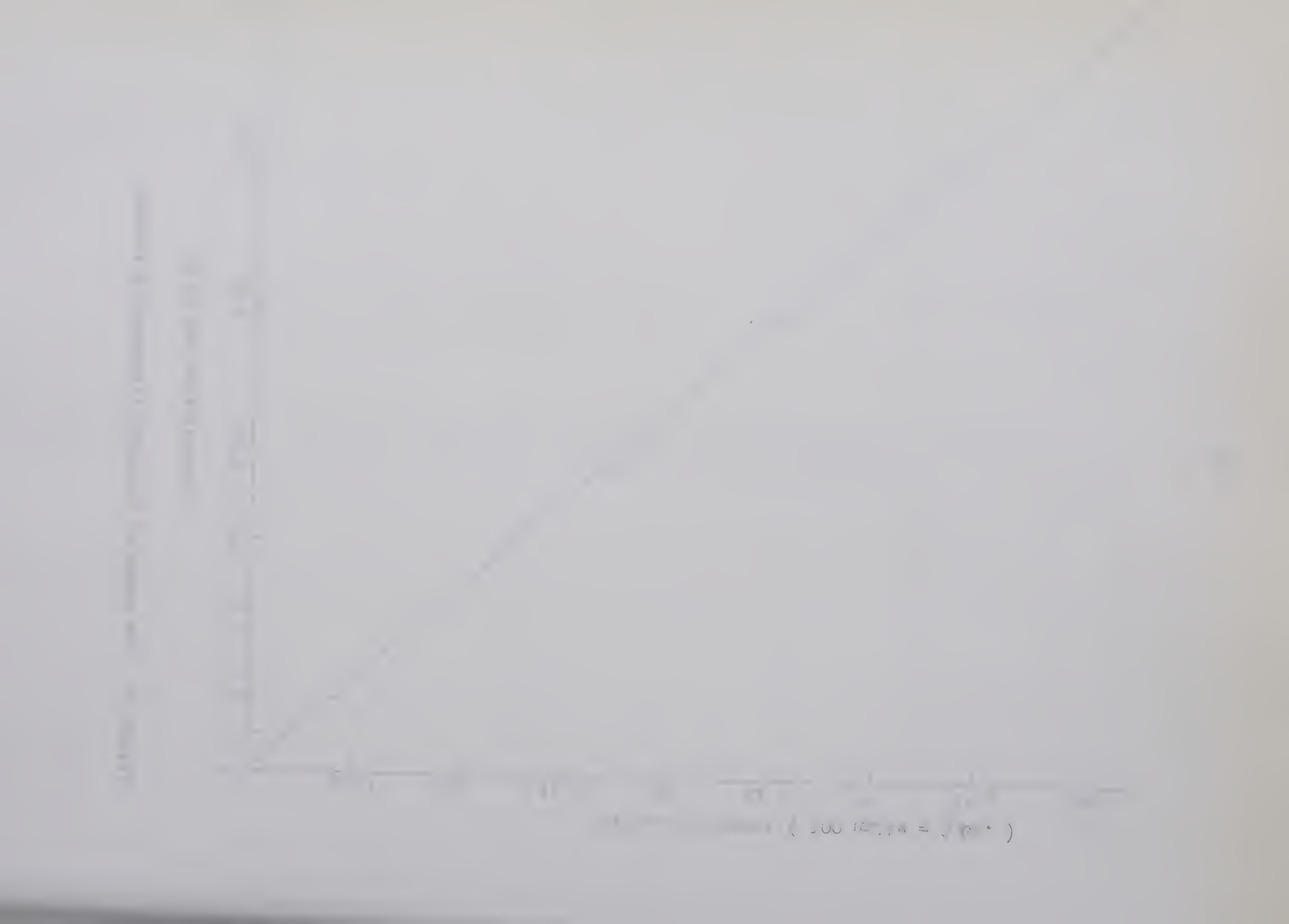


Table LXXX

Comparison of the Microbiological and Polarographic Measurement of Triacetyl Oleandomycin

Temperature of Heating 0° C	Average Diffusion current 100 Units = 3 µa.	Average Diameter of zone of inhibition mm.	Correlation Coefficient r
100	55	10	
110	55	10	
120	55	10	0.878*
130	54	9	
140	50	9	
150	45	8	95% = 0.878 99% = 0.959

* Significant at 95% level.

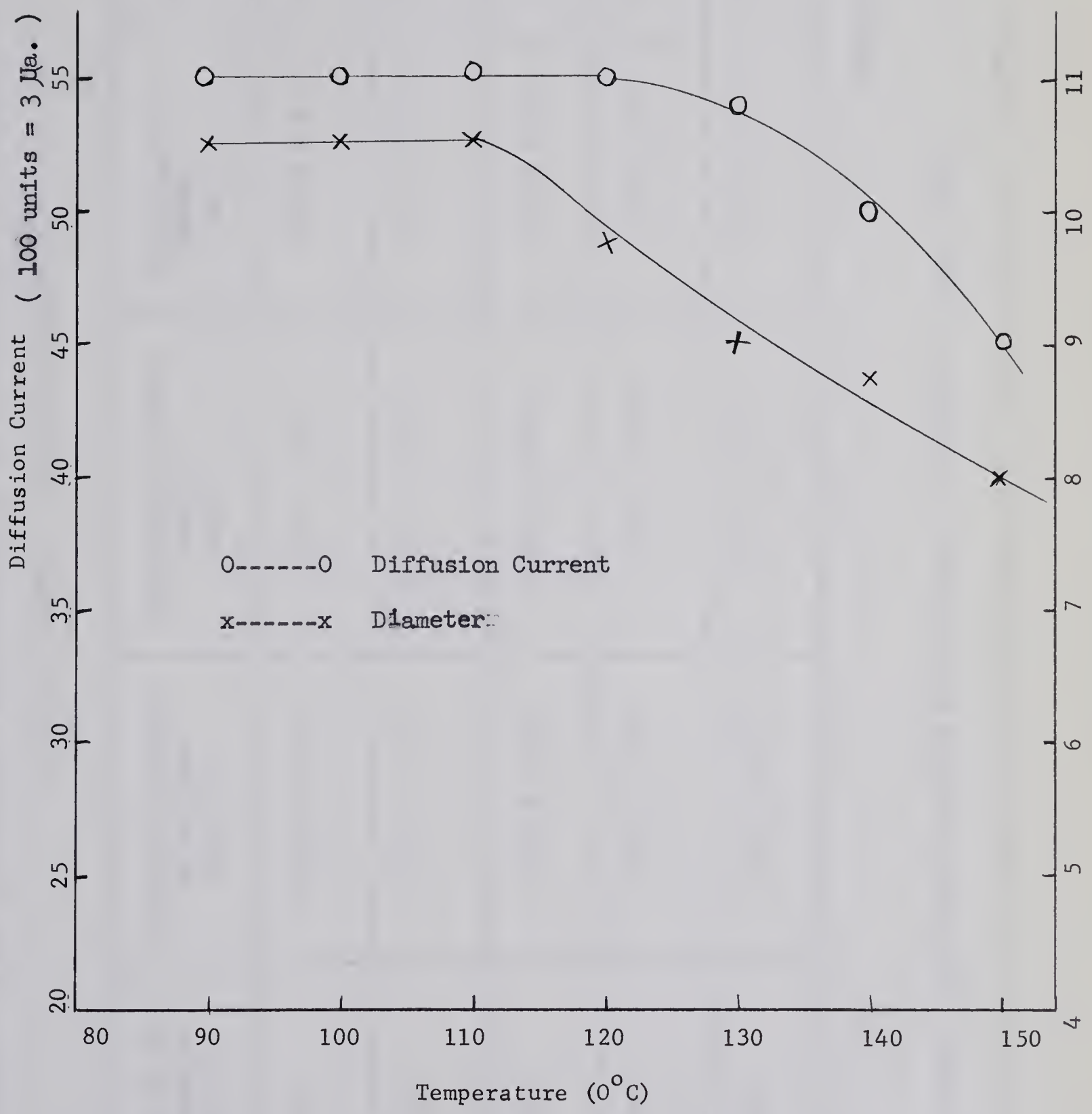


Figure 31. The comparison of microbiological and polarographic measurement of Triacetyl Oleandomycin during degradation.

(10)

Table LXXXI

Results on the Polarographic Determination of the Concentration of Oleandomycin in Olicin (Roerig - 100 mg./capsule)

Amount of the preparation mg./10 ml.	Triacetyl Oleandomycin found mg./10 ml.	Equivalent Oleandomycin mg./10 ml.	Oleandomycin/capsule mg.	% Stated Concentration
0.125	0.057 ± 0.001	0.048 ± 0.001	250.6 ± 4.2	100.2 ± 1.7
0.250	0.114 ± 0.002	0.097 ± 0.002	250.9 ± 4.2	100.4 ± 1.7
0.375	0.169 ± 0.004	0.144 ± 0.003	248.2 ± 5.4	99.3 ± 2.1
0.500	0.228 ± 0.005	0.193 ± 0.004	250.6 ± 5.7	100.3 ± 2.3
0.750	0.343 ± 0.006	0.291 ± 0.005	251.5 ± 4.3	100.6 ± 1.7

Average = 100.2

Individual experimental results are presented in Appendix I, Table XXVII.

Each result is an average of five determinations ± Standard Deviation of the Mean.

Table LXXXII

Results on the Polarographic Determination of the Recovery of Added Triacetyl Oleandomycin from Olicin.

Amount of the preparation mg./10 ml.	Triacetyl Oleandomycin (98.7%)				
	Amount added mg.	Total present mg./10 ml.	Total found mg./10 ml.	Amount recovered mg.	% Recovery
0.25 mg ≡ 0.096 mg. Oleandomycin ≡ 0.114 mg. Triacetyl Oleandomycin ≡ 0.115 mg. Triacetyl Oleandomycin (as 98.7%)	0.05	0.165	0.166 ± 0.001	0.050 ± 0.001	100.0 ± 2.6
	0.10	0.215	0.215 ± 0.002	0.100 ± 0.002	100.0 ± 1.9
	0.15	0.265	0.264 ± 0.003	0.149 ± 0.003	99.3 ± 1.9
	0.20	0.315	0.315 ± 0.003	0.200 ± 0.003	100.0 ± 1.4
	0.25	0.365	0.365 ± 0.005	0.249 ± 0.005	99.7 ± 1.9

Average = 99.8

Individual experimental results are presented in Appendix II, Table XXVII.

Each result is an average of five determinations ± Standard Deviation of the Mean.

could be found in the literature. A brownish-white powder, bacitracin was found to be soluble in water to give a viscous solution which produced a considerable amount of foaming during deaeration. It gave two waves in the pH range of 3.00 to 11.00. The waves below pH 4.50 were not well separated. Cobalt chloride, if used as the supporting electrolyte, gave distinctly separated waves, but they were small; and at alkaline pH the reagent precipitated out. Phosphate buffer at a pH of 9.80 was found most suitable and 0.20 ml of gelatin (0.1%) per 10 ml of the solution was used for better results. The half-wave potentials were -1.22 and -1.54 v. for the two waves under these conditions. The second wave was better for quantitative measurements than the first wave. The results are shown in Table LXXXIII and Figure 32.

IX. Polymyxin B sulphate

There has not been any reported work on a polarographic method for the determination of polymyxin B sulphate. This antibiotic was not completely soluble in water and the addition of a few drops of 3 M HCl was necessary to bring about complete solution. With potassium chloride as the base electrolyte, polymycin B sulphate gave two reduction waves from pH 3.00 to 5.00 after which it gave a single wave up to pH 8.00. The shape of the wave was not very good. From pH 8.50 upward there were again two waves, the second wave losing its peak at pH 10.00. A pH of 6.40, using potassium chloride as the base electrolyte, was found to be most suitable for measurement of the single wave. Gelatin in the usual concentration was used for better results. The

Table LXXXIII

Data on the Diffusion Currents of Second Wave of Bacitracin in Phosphate Buffer at pH 9.80 ± 0.05. $m^{2/3} t^{1/6} = 1.63$ mg. $^{2/3}$ Sec.$^{-1/2}$.

Concentration mg./10 ml.	Diffusion Current 100 units = 3 Ua.	Average ± S.D.M
0.25	2.50 2.50 2.50 2.62 2.50	2.5 ± 0.1
0.50	4.50 4.25 4.50 4.50 4.50	4.4 ± 0.1
0.75	7.00 6.50 6.75 6.75 6.75	6.8 ± 0.2
1.00	8.75 9.00 8.50 8.75 8.75	8.8 ± 0.2
1.50	12.50 12.50 13.00 12.75 12.75	12.7 ± 0.2
2.00	16.50 17.00 17.00 17.25 17.00	17.0 ± 0.3
2.50	20.50 20.75 21.00 21.50 21.00	21.0 ± 0.4
3.00	25.25 25.00 24.50 25.75 24.75	25.0 ± 0.5

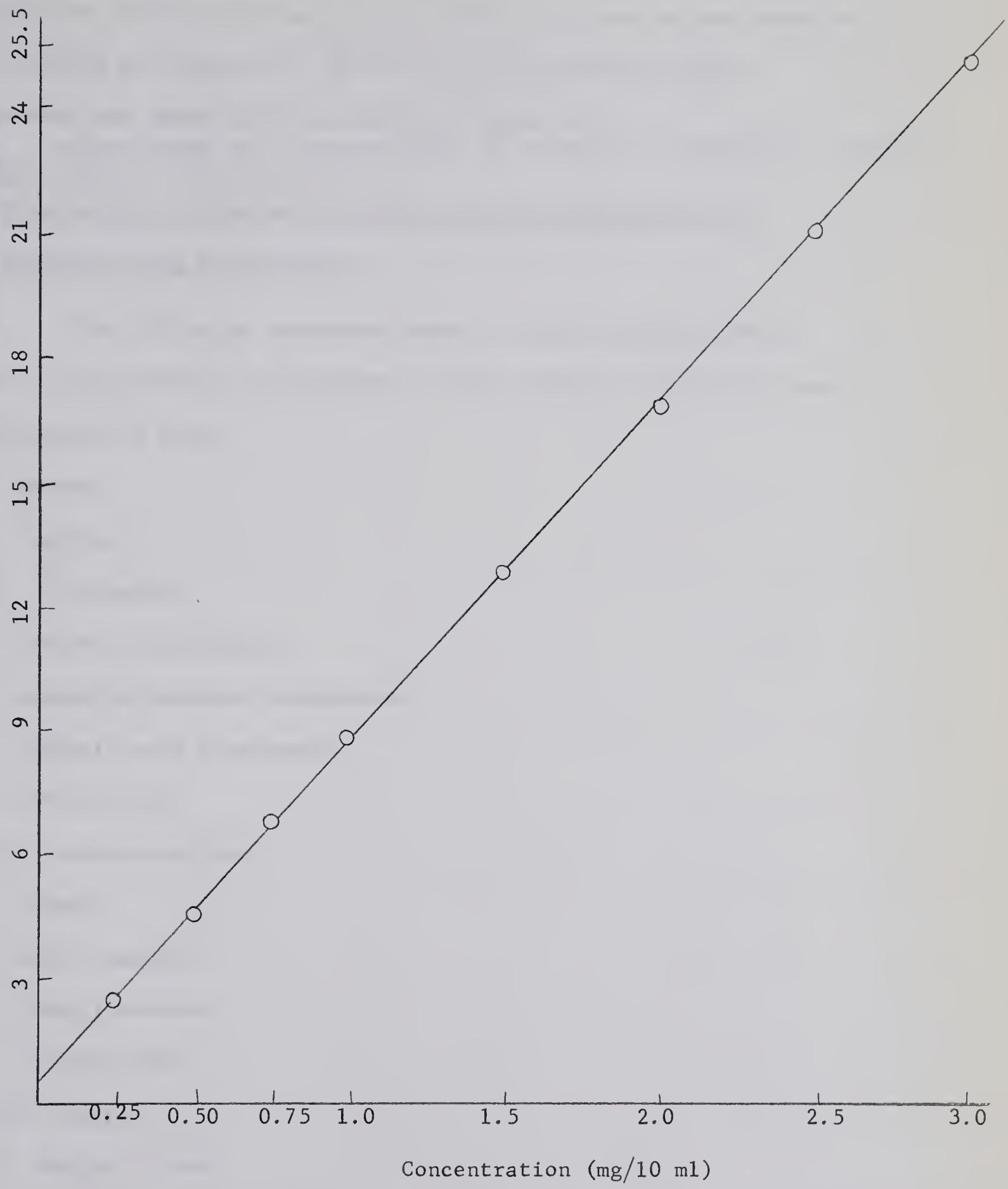
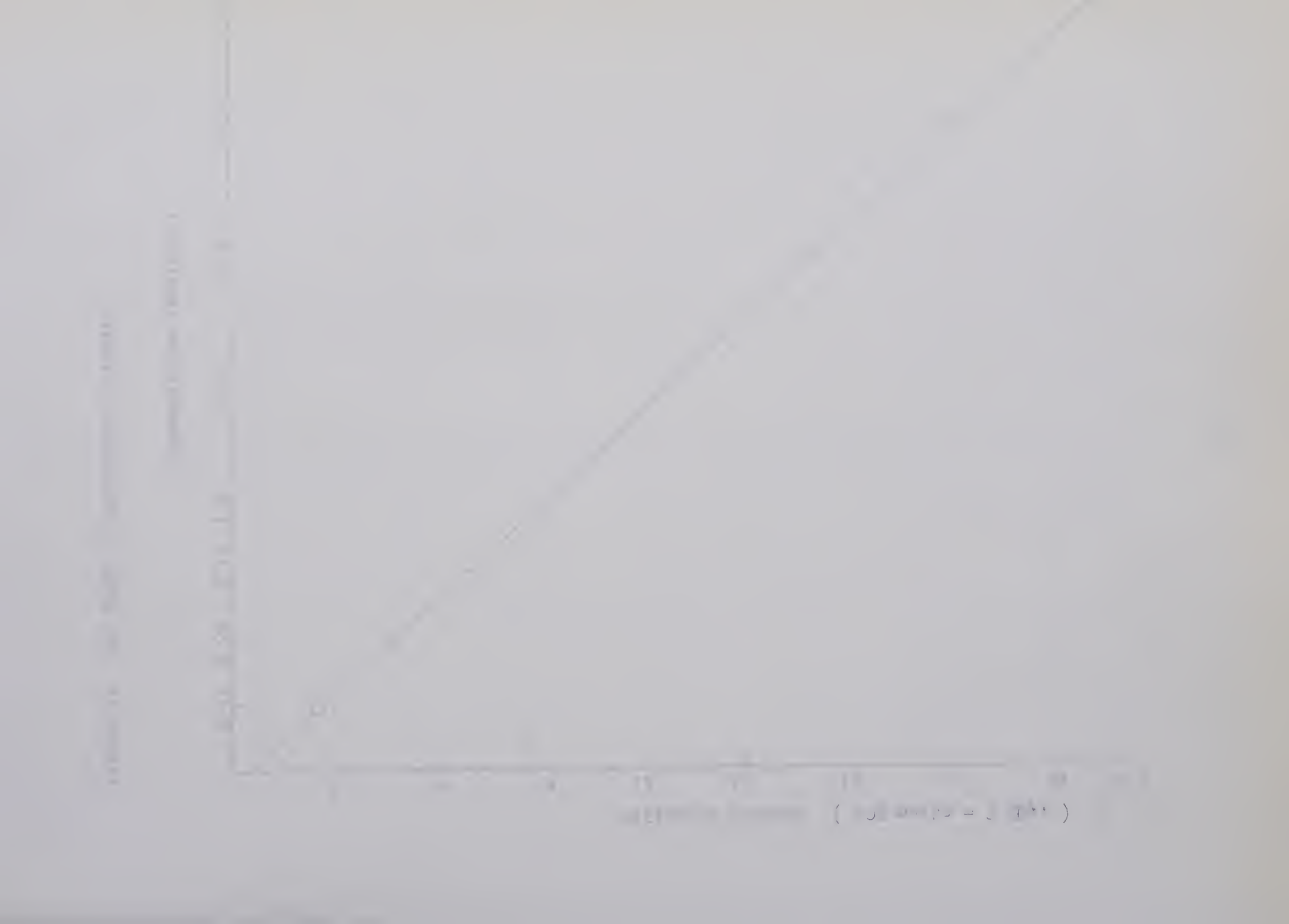


Figure 32. The graph of Bacitracin standard.



E 1/2 value for the wave was -1.64 ± 0.02 v. The results are shown in Table LXXXIV and figure 33. The result of the microbiological comparison are shown in Table LXXXV and figure 34.

Polarography was attempted with no success for novoviocin, nystatin, viocin sulphate and actinomycin D.

X. Polarography of Common Excipients and Other Ingredients of Pharmaceutical Preparations

The following compounds showed no polarographic wave or rise in the conditions and regions in which various antibiotics gave their reduction waves.

(i) Lactose

(ii) Sucrose

(iii) Glucosamine

(iv) N-acetyl glucosamine

(v) Magnesium stearate (suspension)

(vi) Stearic acid (suspension)

(vii) Boric acid

(viii) Calcium sulphate

(ix) Starch

(x) Methyl paraben

(xi) Propyl paraben

(xii) Citric acid

(xiii) Caffeine

(xiv) Sodium citrate

In the following cases there were some rise or a small wave in the regions of antibiotic waves. The proportion in which they were present in the final solution of the pharmaceutical preparations for

Table LXXXIV

Data on the Diffusion Currents of the Single Wave of Polymyxin B Sulphate at pH 6.40 ± 0.05. $m^{2/3} t^{1/6} = 1.62$ mg. $^{2/3}$ Sec. $^{-1/2}$.

Concentration mg./10 ml.	Diffusion Current 100 units = 3 μa.	Average ± S.D.M.
0.125	1.83 1.75 1.75 1.67 1.75	1.8 ± 0.1
0.250	2.83 2.83 3.00 2.83 2.83	2.9 ± 0.1
0.500	5.83 5.50 5.67 5.67 5.50	5.6 ± 0.1
0.750	8.00 8.25 8.50 8.25 8.25	8.2 ± 0.2
1.000	10.25 10.75 10.50 10.50 10.25	10.4 ± 0.2
1.500	15.50 16.00 15.75 16.25 15.50	15.8 ± 0.3
2.000	20.75 21.25 20.50 20.25 20.75	20.7 ± 0.4
2.500	26.00 26.00 25.75 26.75 27.00	26.3 ± 0.5

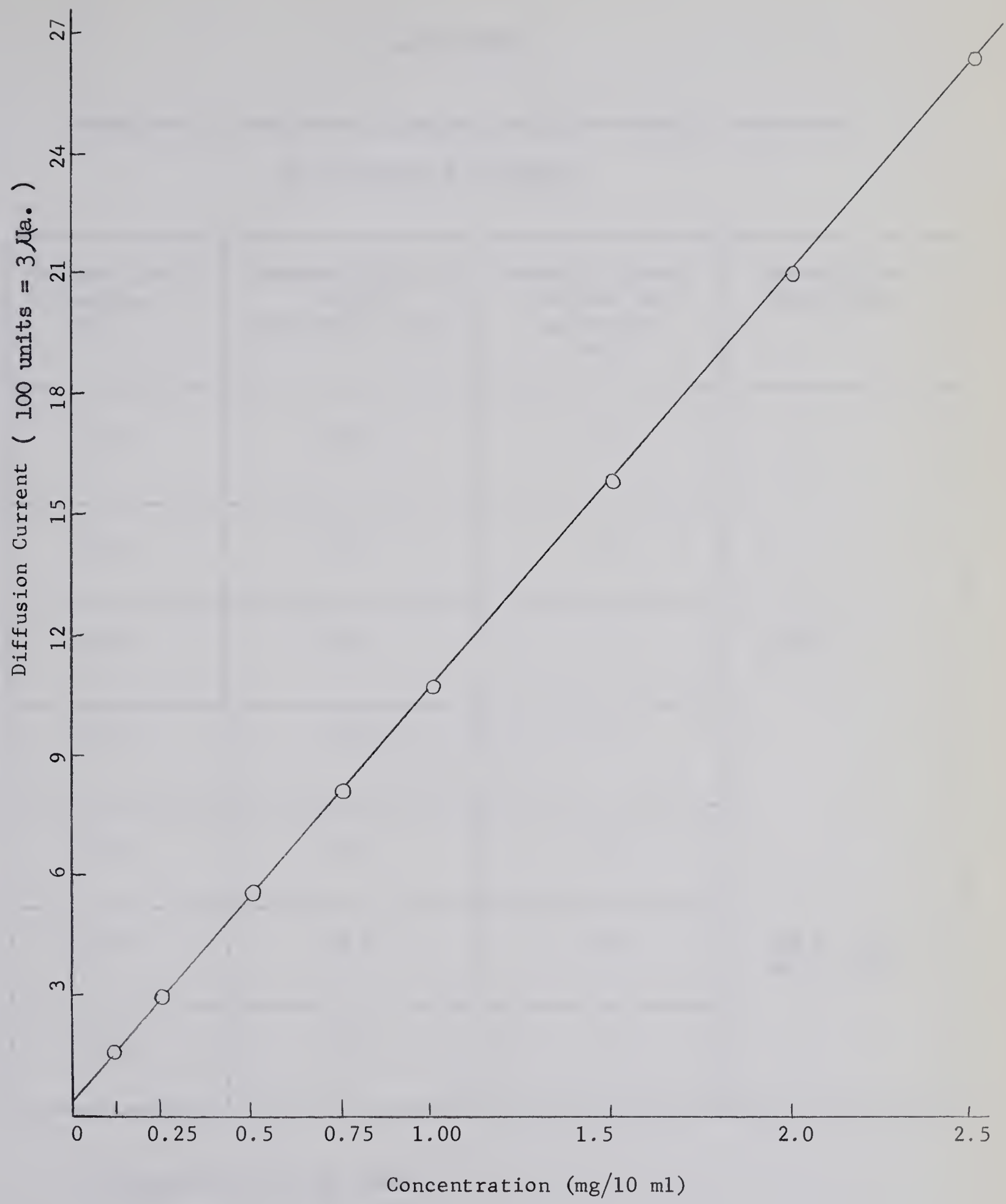


Figure 33. The graph of Polymyxin B Sulphate standard.

Table LXXXV

Comparison of the Microbiological and Polarographic Measurement of Polymyxin B Sulphate.

Temperature of Heating 0° C	Average Diffusion current 100 Units = 3 µa.	Average Diameter of zone of inhibition mm.	Correlation Coefficient r
100	21.8	15	
110	21.6	15	
120	19.2	14	0.95*
125	16.2	14	
130	14.0	13	
140	8.5	10	95% = 0.811 99% = 0.917
150	3.0	4	

* Significant at 99% level.

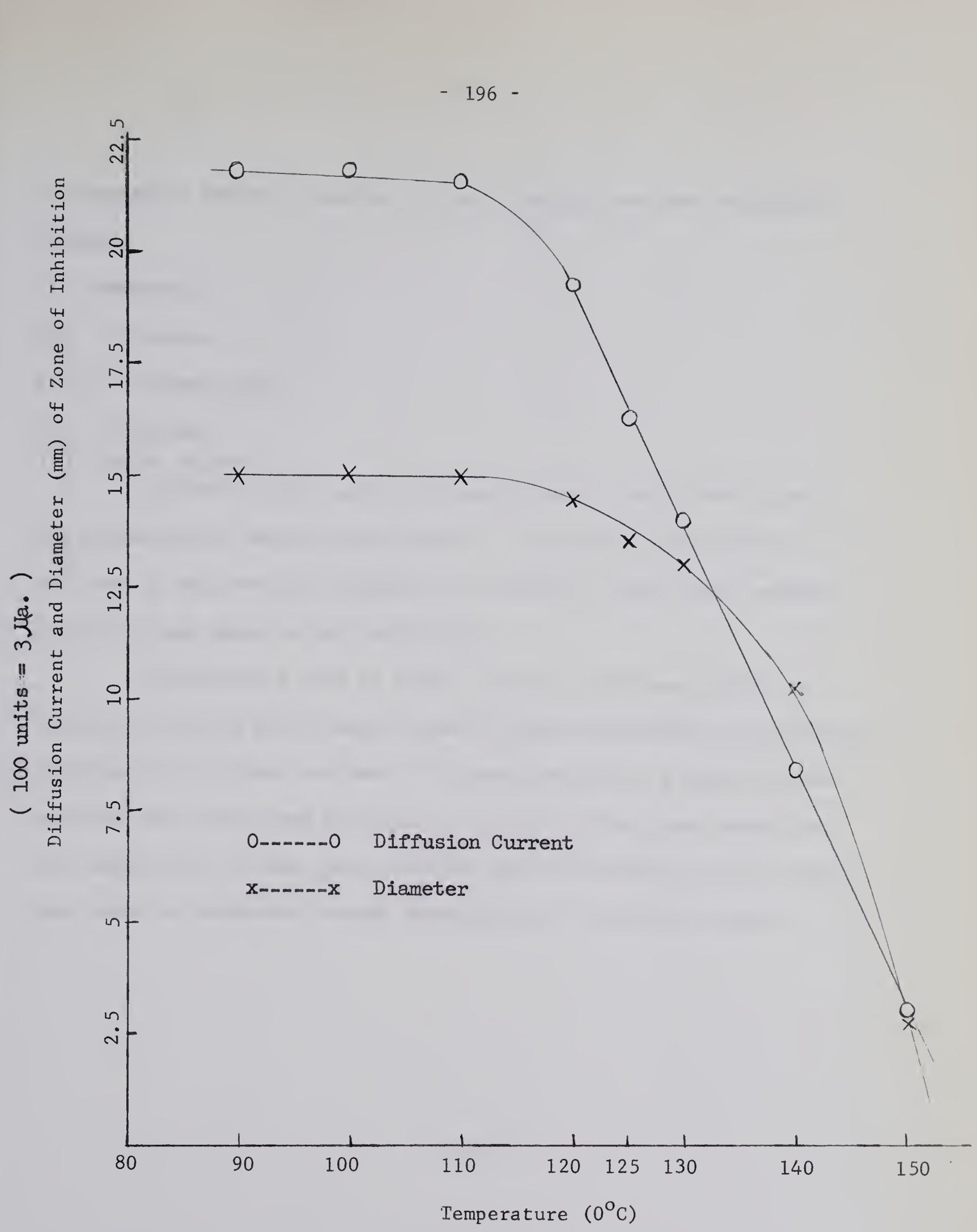


Figure 34. The comparison of microbiological and polarographic measurement of Polymyxin B Sulphate during degradation.

polarographic analysis resulted in very insignificant and negligible effects.

(i) Phenacetin

(ii) Xylocaine

(iii) Sulfamethizole

(iv) Benzocaine

(v) Benzyl alcohol

Ascorbic acid gave a pronounced wave. But in many cases the proportion in which it was present, it had very little effect and even if the wave was obtained it occurred at a much less negative potential than those of the antibiotics.

The solvents such as propyl alcohol, propylene glycol, alcohol, etc. which were commonly used for some suspensions and injectable solutions did not give any wave. In some cases where a small increase occurred, the effect was very small since the solvent was present as only about 0.01% in the final solution used for analysis, and in every case could be corrected through determination of residual current.

V

DISCUSSION

The polarographic method has been applied to the determination of antibiotics. It has been found satisfactory for the determination of tetracycline HCl, tetracycline amphoteric, Cl-tetracycline HCl, demethyl chlorotetracycline HCl, terramycin HCl, terramycin amphoteric, Ca-diterramycin, streptomycin sulphate, streptohydrazid, chloramphenicol, neomycin sulphate, procaine penicillin G, potassium penicillin G, erythromycin, erythromycin ethyl succinate, oleandomycin phosphate, triacetyl oleandomycin, bacitracin and polymyxin B sulphate. It was found unsuitable or impractical for dihydrostreptomycin sulphate, novobiocin, nystatin, viocin sulphate and actinomycin D in the tests carried out under the conditions used.

In most cases, the degree of accuracy was found to be between 1.25 to 2.75%. The sensitivity was found to be as low as 5γ/ml in most of the antibiotics and as low as 2.5γ/ml in the case of chloramphenicol, erythromycin ethyl succinate and triacetyl oleandomycin. These degrees of sensitivity are very favourable when compared with most chemical methods.

I. Tetracyclines:

Tetracycline HCl could be analysed with a good degree of accuracy. In the case of pharmaceutical preparations only the presence of metallic ions such as magnesium chloride as in Tetrex I.M. (page 66) posed any problem. It was found possible to counteract this problem by the addition of EDTA. The minor effects of various other components, if any, could be eliminated by running a blank of the components. The recovery of the pure antibiotics from the respective pharmaceutical preparations was found to be very good (99.00 to 100.50%).

Cl-tetracycline and DMCT were measurable with similar ease and to the same degree of accuracy. There was no problem in those pharmaceutical preparations of these antibiotics which were analysed. Although solubility of the active component was more difficult in Achromycin capsules the addition of a few drops of 3 M HCl brought this into complete solution. To ensure complete solution of the antibiotics in all preparations a few drops of 3 M HCl could be added.

Oxytetracycline HCl and Ca-diterramycin were likewise analysed with a good degree of accuracy and to the same degree of sensitivity. Ca-diterramycin was analysed as oxytetracycline after solution in an acid media of pH 3.00 using HCl. The presence of calcium ion caused similar interference to that encountered in Tetrex I.M. and the addition of EDTA was necessary to remove this interference.

There was no problem encountered in the assay of pharmaceutical preparations. Either there were no waves due to the other components present in the region where oxytetracycline was measured or in cases where there were waves the effect was insignificant since it was present in very small proportion in the final solution analysed and could be subtracted from the diffusion current. This interference was corrected by the use of a blank containing the known components.

II. Streptomycins:

Streptomycin was analysed in tetraethyl ammonium hydroxide in preference to sodium hydroxide as it is more stable in the former. This particular antibiotic gave the best results of all the antibiotics studied. No pharmaceutical preparation was available for further examination of

this method. However, it is strongly felt that there would be no difficulty in employing this method for the assay of pharmaceutical preparations.

Dihydrostreptomycin showed a wave throughout the pH range of 6.00 to 12.00, but since the wave was obtained at a comparatively more negative potential the wave could not be fully developed due to the interference of the wave due to the base electrolyte.

Streptohydrazid, on the other hand, could be analysed for the hydrazid moiety using the same base electrolyte as was used for streptomycin and at the same pH. The wave for streptomycin was not present at the pH used for analysis.

III. Chloramphenicol:

Chloramphenicol gave best results in biphthalate buffer. In both phosphate and borate buffer the residual current increased slowly making the measurements of the diffusion current difficult.

Since the assay of many of the pharmaceutical preparations has already been reported (147) analysis on only one preparation which had not been previously done was attempted. Chloromycetin ophthalmic could be analysed easily under the similar experimental conditions used for pure chloramphenicol. An attempt was made to analyse chloromycetin cream, without going through the lengthy extraction procedure reported by Summa (147). The low solubility of the ointment base in the aqueous media was a problem. It is felt that if the assay conditions could be made non-aqueous the extraction procedure could be avoided and a satisfactory assay carried out. This was not ascertained.

IV. Neomycin Sulphate:

Neomycin sulphate was analysed polarographically after it was hydrolysed to furfural with mineral acid on a boiling water bath. Furfural was reduced readily in a polarographic cell and accurate measurement was possible without separating this compound from other hydrolysis products. This is a decisive advantage over the spectrophotometric method of Morgan et al. (71) in which chromatographic separation of the furfural obtained from neomycin was found necessary prior to its determination.

V. Erythromycins:

Erythromycin, for which there have been fewer analysis methods reported, could be analysed satisfactorily by polarography.

Erythromycin ethyl succinate gave a reduction wave similar to that of erythromycin under similar conditions but the wave was obtained at less negative potential.

Under identical conditions there was no difficulty in applying the polarographic method of analysis to pharmaceutical preparations of this antibiotic. The presence of polyethylene glycol as in Erythrocin I.M. (page 161) was found to shift the wave of the antibiotic to a more negative potential. If the same proportion of this solvent was added during the preparation of the standard solution, the waves were obtained at the same potentials thus facilitating the measurement of erythromycin ethyl succinate in Erythrocin I.M.

VI Penicillins:

Penicillins were analysed by an indirect method after the reduction of the nitrosated hydrolysed product (149). Some improvements were found possible and are reported herein.

Since the method still involved lengthy steps of hydrolysis, nitrosation, standing etc. it does not have any particular advantage over the existing lengthy chemical methods of measurements (83-96).

However, procaine penicillin G could be measured by measuring directly the procaine part polarographically. A well defined reduction wave was obtained which corresponded to that of pure procaine under similar conditions and could be measured quantitatively.

VII. Oleandomycins:

Oleandomycin phosphate and triacetyl oleandomycin for which there have been few quantitative measurement methods reported, could both be analysed polarographically. The two antibiotics could not be analysed in the same environment of pH and buffer conditions since acetyl oleandomycin behaved differently than oleandomycin phosphate and the best waves in the two cases were obtained at two different pH's. In both cases, however, the quantitative results obtained were acceptable with a good degree of accuracy.

VIII. Bacitracin:

A gravimetric method is the only chemical method available for the measurement of bacitracin (101) other than microbiological methods. In phosphate buffer of pH 9.80 ± 0.05 it was found possible to assay bacitracin polarographically with an acceptable degree of accuracy.

IX. Polymyxin:

Besides the microbiological method there has been only one other method for the measurement of polymyxin antibiotic reported. This is a gravimetric method and can be applied to macroquantities (101).

This antibiotic could be analysed satisfactorily in the semi-micro range and with a good degree of accuracy by the polarographic method. A pH of 6.40 ± 0.10 and the use of potassium chloride as the base electrolyte were found to be most suitable.

X. Microbiological Comparison:

The correlation between diffusion current, and therefore the amount of antibiotic as determined by the polarographic method, and the activity of the antibiotic, as determined by the microbiological method on the antibiotic samples heated to different temperatures, was very good.

In some cases, however, the products obtained by heating antibiotics to elevated temperatures had some effect on the polarographic waves of the antibiotics. In the case of oxytetracycline amphoteric and Ca-diterramycin the residual current was somewhat larger than normal due to a wave appearing before the waves of oxytetracycline in the heated preparations. This did not have any significant effect on the waves of the antibiotics and it was possible to determine the diffusion current accurately. The same kind of effect was observed in the case of oxytetracycline HCl but at higher temperatures (above 120°C) than the previous two forms of this antibiotic.

No such wave prior to the waves of tetracycline was observed even at such temperatures as 150°C where there was an appreciable amount

of degradation.

Such a comparison of the methods could not be made for chloramphenicol since this antibiotic did not show any decrease in activity or diffusion current until at 150°C when it melted and was converted into a dark mass which was not soluble in a variety of solvent systems. This comparison was also not possible for bacitracin since a sensitive organism was not readily available. Such comparison was not attempted for the antibiotics of the penicillin group since the polarographic method of analysis involved hydrolysis of the active molecules, and if hydrolysis were carried out at gradual intervals, the procaine part would have been measured together with the penicillin fraction (page 169).

In the case of neomycin there was no breakdown by heating alone even up to 150°C and the comparison was done by the addition of hydrochloric acid (2.50 ml of 37% acid) and heating on a water bath at 90°C for different lengths of time. As the time of hydrolysis was increased the diffusion current increased and the activity decreased as expected until all the antibiotic was hydrolysed (Figure 21).

The correlation coefficient was not significant for streptohydrazid since the polarographic method measured the isoniazid part whereas the microbiological method measured the streptomycin and probably also the isoniazid part of the product.

VI

SUMMARY AND CONCLUSION

1. The polarographic method has been successfully applied to the determination of the following antibiotics:
tetracycline hydrochloride, tetracycline amphoteric, chlorotetracycline hydrochloride, demethyl chlorotetracycline hydrochloride, oxytetracycline hydrochloride, oxytetracycline amphoteric, calcium diterramycin, streptomycin sulphate, streptohydrazid, chloramphenicol, neomycin sulphate, erythromycin, erythromycin ethyl succinate, procaine penicillin G, potassium penicillin G, oleandomycin phosphate, triacetyl oleandomycin, bacitracin and polymyxin B sulphate.

2. The polarographic method has been used for the first time as far as it has been possible to determine for the assay of tetracycline amphoteric, demethyl chlorotetracycline hydrochloride, calcium diterramycin, erythromycin, erythromycin ethyl succinate, oleandomycin phosphate, triacetyl oleandomycin, bacitracin and polymyxin B sulphate.

3. The direct polarographic determination of procaine penicillin G by measuring the procaine part without nitrosation has also not been reported previously to the best of our knowledge.

4. In all instances definite conditions were established and verified for the satisfactory analysis of the particular antibiotic.

5. The polarographic method was not successful for measurement of dihydrostreptomycin, novobiocin, nystatin, viocin sulphate and actinomycin D under the conditions used.

6. The degree of error in most of the cases was found to be between 1.25 and 2.75% which was considered as very acceptable.

7. The sensitivity was found to be as low as 5 γ/ml in most of the antibiotics and as low as 2.5γ/ml in the cases of chloramphenicol, erythromycin ethyl succinate and triacetyl oleandomycin.

8. Good correlation was obtained between the polarographic and microbiological methods of measurements of the active antibiotics. Chloramphenicol could not be degraded by stages and comparison was not possible. Microbiological comparison was also not possible for neomycin sulphate and penicillin antibiotics since the polarographic method of measurement involved hydrolysis of the active components in these two cases. Bacitracin could not be compared since the micro-organism sensitive to this antibiotic was not readily available.

9. The polarographic method was successfully applied to the quantitative evaluation of antibiotics present in 27 different commercial pharmaceutical preparations.

10. The method was found to be simple and very rapid. Analysis was possible using the preparation without prior extraction or other analytical procedures for separation other than simple dilution.

11. The percentage potency as determined by the polarographic method in all the cases of pharmaceutical preparations was within the specified limits of the U.S.P. and B.P.

12. The percentage recovery of pure antibiotics from their respective pharmaceutical preparations as determined by the polarographic method was very good and in the region of 99.00 to 100.75%.

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Appendix I

Polarographic determination of the potency of the antibiotics in pharmaceutical preparations.

Table I

Tetracyn Capsule

Weight of preparation mg	Tetracycline HCl 99.03%		Pharmaceutical Preparation		Tetracycline HCl found mg	Tetracycline HCl found/ capsule mg	% Potency
	mg/10 ml	Average diffusion current	mg/10 ml	Diffusion current			
668.7	0.202	7.30	0.50	7.25	0.1987	267.97	107.19
				7.00	0.1918	258.66	103.46
				7.50	0.2055	277.14	110.86
684.7				7.25	0.1987	267.97	107.19
				7.00	0.1918	258.66	103.46
684.7	0.303	10.55	0.75	10.50	0.2986	268.46	107.38
660.7				10.75	0.3058	274.93	109.97
683.3				10.25	0.2915	262.07	104.83
				10.25	0.2915	262.07	104.83
676.2				10.50	0.2986	268.46	107.38
669.0	0.404	14.10	1.00	14.00	0.3972	267.83	107.13
660.4				13.75	0.3902	263.11	105.24
678.6				14.50	0.4113	277.40	110.96
				14.25	0.4043	272.62	109.05
676.6				14.00	0.3972	267.83	107.13
	0.606	21.00	1.50	21.25	0.6073	273.00	109.20
				20.75	0.5930	266.57	106.63
				21.50	0.6144	276.19	110.48
				20.50	0.5858	263.33	105.33
				21.00	0.6001	269.76	107.90
Average = 674.29 ± 9.20	0.808	27.45	2.00	26.50	0.7725	260.44	104.18
				26.75	0.7798	262.90	105.16
				28.00	0.8162	275.17	110.07
				27.25	0.7943	267.79	107.12
				27.25	0.7943	267.79	107.12
							Average = 107.17±2.26

Table II

Signemycin Capsule

Weight of preparation mg	Tetracycline HCl 99.03%		Pharmaceutical Preparation		Tetracycline HCl found mg	Tetracycline HCl found/ capsule mg	% Potency
	mg/10 ml	Average diffusion current	mg/10 ml	Diffusion current			
398.9	0.11	4.30	0.25	4.25	0.1077	173.63	103.97
				4.50	0.1140	183.79	110.05
395.7				4.25	0.1077	173.63	103.97
				4.25	0.1077	173.63	103.97
				4.25	0.1077	173.63	103.97
417.3	0.22	8.00	0.50	8.00	0.2179	175.65	105.18
397.0				8.25	0.2247	181.13	108.46
404.2				7.75	0.2111	170.17	101.90
				8.00	0.2179	175.65	105.18
417.1				7.75	0.2111	170.17	101.90
398.3	0.44	15.20	1.00	15.50	0.4443	179.07	107.23
				15.00	0.4300	173.31	103.78
407.1				14.75	0.4228	170.40	102.04
				15.25	0.4372	176.21	105.51
396.4				14.75	0.4228	170.40	102.04
398.4	0.66	22.50	1.50	22.00	0.6391	171.72	102.83
				21.75	0.6318	169.76	101.65
				23.00	0.6681	179.51	107.49
				22.25	0.6463	173.65	103.98
Average =				22.50	0.6536	175.62	105.16
403.04 ±	0.88	33.00	2.00	30.75	0.8933	180.02	107.80
8.26				29.50	0.8569	172.68	103.40
				30.25	0.8787	177.08	106.04
				29.00	0.8424	169.76	101.65
				29.00	0.8424	169.76	101.65

Average =
104.43±2.37



Table III

Tetrosol

Weight of preparation mg	Tetracycline HCl 99.03%		Pharmaceutical Preparation		Tetracycline HCl found mg	Tetracycline HCl found/ tablet mg	% Potency
	mg/10 ml	Average diffusion current	mg/10 ml	Diffusion current			
518.1	0.24	8.70	0.50	8.75	0.2390	248.15	99.26
				8.50	0.2322	241.09	96.44
523.2				8.50	0.2322	241.09	96.44
516.4				8.75	0.2390	248.15	99.26
				8.75	0.2390	248.15	99.26
521.1	0.36	12.75	0.75	12.75	0.3565	246.77	98.71
				12.50	0.3495	241.92	96.77
517.0				12.75	0.3565	246.77	98.71
				12.25	0.3425	237.08	94.83
				12.75	0.3565	246.77	98.71
	0.48	16.80	1.00	17.00	0.4810	249.72	99.89
				16.50	0.4668	242.34	96.94
Average =				16.70	0.4739	246.03	98.41
				16.00	0.4527	235.02	94.01
519.16 ±				16.25	0.4598	238.71	95.48
2.89	0.72	24.50	1.50	24.00	0.6985	241.76	96.70
				24.75	0.7276	251.82	100.73
				24.25	0.7057	244.25	97.70
				24.00	0.6985	241.76	96.70
				23.50	0.6839	236.70	94.68
	0.96	35.65	2.00	36.25	0.9666	250.92	100.37
				34.25	0.9133	237.07	94.83
				35.50	0.9467	245.74	98.30
				35.25	0.9400	244.00	97.60
				34.75	0.9267	240.55	96.22

Average = 97.48±1.87

Table IV

Achromycin Capsule

Weight of preparation mg	Tetracycline HCl 99.03%		Pharmaceutical Preparation		Tetracycline HCl found mg	Tetracycline HCl found/ capsule mg	% Potency
	mg/10 ml	Average diffusion current	mg/10 ml	Diffusion current			
377.0	0.17	6.10	0.25	6.00	0.1656	255.79	102.32
				5.75	0.1587	245.13	98.05
391.9				6.00	0.1656	255.79	102.32
				6.00	0.1656	255.79	102.32
384.3				5.75	0.1587	245.13	98.05
	0.34	12.00	0.50	12.00	0.3367	260.03	104.01
393.3				11.75	0.3297	254.62	101.85
387.0				11.25	0.3157	243.81	97.52
				12.00	0.3367	260.03	104.01
389.9				11.50	0.3227	249.21	99.68
390.1	0.51	17.55	0.75	17.25	0.4964	255.57	102.23
				17.75	0.5108	262.98	105.19
384.4				16.75	0.4820	248.16	99.26
				17.25	0.4964	255.57	102.23
375.2				17.00	0.4992	257.01	102.80
388.3	0.68	23.25	1.00	23.00	0.6662	257.25	102.90
				22.00	0.6372	246.05	98.42
Average =				23.25	0.6734	260.03	104.01
				22.50	0.6517	251.65	100.66
386.14 ±				22.25	0.6444	248.83	99.53
6.05	1.02	34.70	1.50	34.00	0.9897	254.78	101.91
				33.00	0.9606	247.29	98.92
				34.75	1.0116	260.42	104.17
				33.75	0.9824	252.90	101.16
				33.00	0.9606	247.29	98.92

Average =
101.30±2.25

Table V

Achromycin Syrup

Volume of preparation	Tetracycline HCl 99.03%		Pharmaceutical Preparation		Tetracycline HCl found	Tetracycline HCl found/ml	%
	mg/10 ml	Average diffusion current	ml/10 ml	Diffusion current	mg	mg	Potency
2 fl. oz. bottles	0.125	4.55	0.005	4.50	0.1224	24.48	97.92
				4.50	0.1224	24.48	97.92
				4.75	0.1292	25.84	103.36
				4.50	0.1224	24.48	97.92
				4.50	0.1224	24.48	97.92
	0.250	8.50	0.010	8.50	0.2476	24.67	99.04
				8.75	0.2549	25.49	101.96
				8.25	0.2403	24.03	96.12
				8.50	0.2476	24.67	99.04
				8.75	0.2549	25.49	101.96
	0.500	17.00	0.020	17.00	0.4952	24.76	99.04
				17.50	0.5097	25.48	101.92
				16.75	0.4879	24.40	97.60
				17.00	0.4952	24.76	99.04
				17.25	0.5024	25.12	100.48
	0.750	25.05	0.030	25.50	0.7561	25.20	100.80
				25.00	0.7412	24.71	98.84
				24.75	0.7338	24.46	97.84
				25.75	0.7635	25.45	101.80
				25.75	0.7635	25.45	101.80
	1.000	33.75	0.040	33.50	0.9830	24.58	98.32
				34.00	0.9976	24.94	99.76
				34.25	1.0050	25.12	100.48
				33.25	0.9756	24.39	97.56
				34.50	1.0123	25.31	101.24

Average = 99.59±1.87

Table VI

Achrocidin Tablet

Weight of preparation mg	Tetracycline HCl 99.03%		Pharmaceutical Preparation		Tetracycline HCl found mg	Tetracycline HCl found/ tablet mg	% Potency
	mg/10 ml	Average diffusion current	mg/10 ml	Diffusion current			
789.6	0.20	7.00	1.25	7.00	0.1980	124.10	99.28
				7.25	0.2051	128.55	102.84
799.1				7.00	0.1980	124.10	99.28
				7.00	0.1980	124.10	99.28
				7.25	0.2051	128.55	102.84
768.9	0.40	13.75	2.50	13.75	0.3961	124.13	99.30
783.1				13.50	0.3889	121.87	97.50
787.1				14.00	0.4033	126.39	101.11
				14.25	0.4105	128.64	102.91
789.7				13.75	0.3961	124.13	99.30
764.8	0.60	20.40	3.75	20.50	0.5971	124.75	99.80
				20.75	0.6044	126.27	101.02
791.8				20.25	0.5898	123.22	98.58
802.4				21.00	0.6117	127.80	102.24
758.0				20.25	0.5898	123.22	98.58
	0.80	27.25	5.00	27.50	0.7995	125.27	100.22
				28.00	0.8140	127.55	102.04
Average =				27.00	0.7850	123.00	98.40
				26.75	0.7777	121.86	97.49
783.45 ±				27.50	0.7995	125.27	100.22
14.18	1.00	33.80	6.25	33.75	0.9888	123.95	99.16
				34.50	1.0108	126.70	101.36
				35.00	1.0254	128.53	102.82
				34.00	0.9962	124.87	99.90
				33.25	0.9742	122.12	97.70

Average = 100.13±1.75

Table VII

Achrocidin Compound Syrup

Volume of preparation	Tetracycline HCl 99.03%		Pharmaceutical Preparation		Tetracycline HCl found	Tetracycline HCl found/ml	%
	mg/10 ml	Average diffusion current	ml/10 ml	Diffusion current	mg	mg	Potency
2 fl. oz. bottles	0.130	5.00	0.005	5.00	0.1287	25.74	102.96
				5.00	0.1287	25.74	102.96
				5.25	0.1352	27.04	108.16
				5.00	0.1287	25.74	102.96
				5.25	0.1352	27.04	108.16
	0.260	9.25	0.010	9.50	0.2644	26.44	105.76
				9.25	0.2575	25.75	103.00
				9.25	0.2575	25.75	103.00
				9.75	0.2714	27.14	108.56
				9.25	0.2575	25.75	103.00
	0.520	17.75	0.020	17.75	0.5150	25.75	103.00
				18.25	0.5295	26.48	105.92
				18.00	0.5222	26.11	104.44
				18.50	0.5367	26.84	107.36
				17.50	0.5077	25.38	101.52
	0.780	26.50	0.030	27.00	0.7870	26.23	104.92
				27.50	0.8016	26.72	106.88
				26.50	0.7724	25.75	103.00
				26.75	0.7797	25.99	103.96
				27.25	0.7943	26.48	105.92
	1.040	35.25	0.040	35.00	1.0226	25.56	102.24
				36.00	1.0518	26.30	105.20
				35.50	1.0372	25.93	103.72
				36.75	1.0737	26.84	107.36
				35.75	1.0445	26.11	104.44

Average = 104.74±2.04

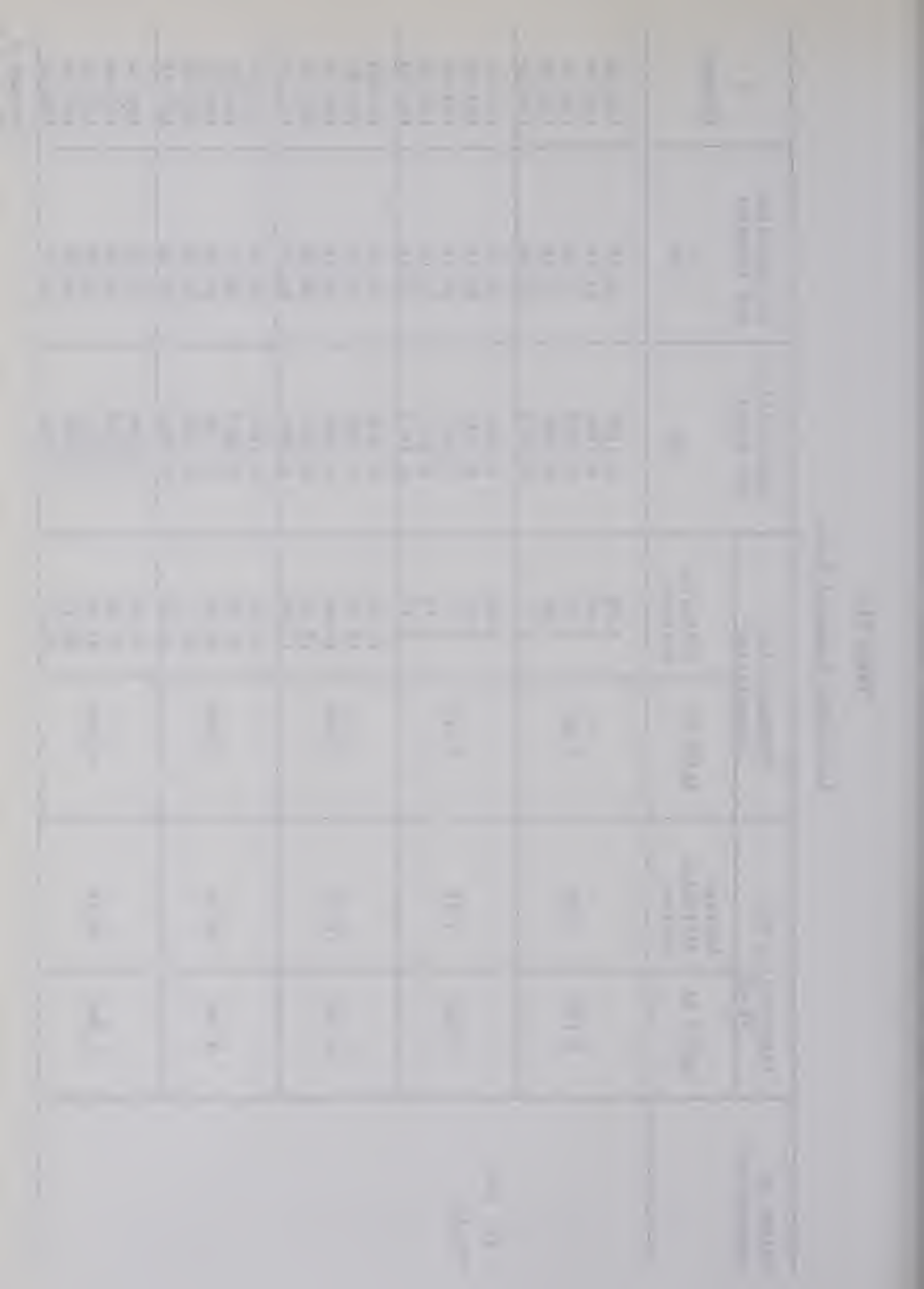


Table VIII

Tetrex Capsule

Weight of preparation mg	Tetracycline HCl 99.03%		Pharmaceutical Preparation		Tetracycline HCl found mg	Tetracycline HCl found/ capsule mg	% Potency
	mg/10 ml	Average diffusion current	mg/10 ml	Diffusion current			
240.1	0.2150	7.85	0.50	7.75	0.2102	104.32	104.32
				8.00	0.2170	107.69	107.69
				7.75	0.2102	104.32	104.32
244.6				8.00	0.2170	107.69	107.69
251.9				8.00	0.2170	107.69	107.69
249.4	0.3225	11.25	0.75	11.25	0.3194	105.67	105.67
				11.00	0.3123	103.32	103.32
253.3				11.50	0.3265	108.02	108.02
				11.50	0.3265	108.02	108.02
249.8				11.25	0.3194	105.67	105.67
245.4	0.4300	15.10	1.00	15.00	0.4230	104.96	104.96
				15.25	0.4301	106.72	106.72
246.3				14.75	0.4160	103.23	103.23
255.8				15.50	0.4371	108.46	108.46
244.8				15.50	0.4371	108.46	108.46
Average = 248.14 ± 4.75	0.6450	22.25	1.50	22.00	0.6316	104.48	104.48
				22.50	0.6459	106.85	106.85
				21.75	0.6244	103.29	103.29
				22.75	0.6531	108.04	108.04
				21.50	0.6172	102.10	102.10
	0.8600	29.30	2.00	29.50	0.8575	106.39	106.39
				28.75	0.8357	103.68	103.68
				30.00	0.8720	108.19	108.19
				29.75	0.8647	107.28	107.28
				29.25	0.8502	105.48	105.48

Average = 106.00±1.97

Table IX

Tetrex I.M.

Weight of preparation mg	Tetracycline HCl 99.03%		Pharmaceutical Preparation		Tetracycline HCl found mg	Tetracycline HCl found/ vial mg	% Potency
	mg/10 ml	Average diffusion current	mg/10 ml	Diffusion current			
897.5	0.125	4.75	0.4375	4.75	0.1238	253.96	101.58
				4.75	0.1238	253.96	101.58
				5.00	0.1303	267.30	106.92
				4.75	0.1238	253.96	101.58
				5.00	0.1303	267.30	106.92
	0.250	9.00	0.875	9.25	0.2544	260.94	104.38
				9.00	0.2476	253.96	101.58
				9.25	0.2544	260.94	104.38
				9.00	0.2476	253.96	101.58
				9.25	0.2544	260.94	104.38
	0.500	17.45	1.750	17.50	0.4966	254.68	101.87
				18.00	0.5108	261.97	104.79
				18.25	0.5178	265.56	106.22
				17.25	0.4895	251.04	100.42
				17.50	0.4966	254.68	101.87
	0.750	25.55	2.625	25.50	0.7412	253.42	101.37
				26.00	0.7558	258.41	103.36
				25.25	0.7340	250.96	100.38
				26.50	0.7703	263.37	105.35
				26.00	0.7558	258.41	103.36
	1.000	34.00	3.500	33.75	0.9830	252.07	100.83
				35.00	1.0194	261.40	104.56
				34.00	0.9903	253.94	101.58
				35.25	1.0267	263.28	105.31
				35.00	1.0194	261.40	104.56

Average =
102.23±2.04

Table X

Azotrex (Capsule)

Weight of preparation mg	Tetracycline HCl 99.03%		Pharmaceutical Preparation		Tetracycline HCl found mg	Tetracycline HCl found/ capsule mg	% Potency
	mg/10 ml	Average diffusion current	mg/10 ml	Diffusion current			
532.6	0.125	4.60	0.50	4.75	0.1278	132.55	106.04
549.9				4.75	0.1278	132.55	106.04
497.7				4.50	0.1211	125.60	100.48
530.8				4.75	0.1278	132.55	106.04
493.4				4.50	0.1211	125.60	100.48
506.6	0.250	9.00	1.00	9.00	0.2476	128.41	102.73
562.8				9.00	0.2476	128.41	102.73
493.3				9.25	0.2544	131.93	105.54
505.7				9.00	0.2476	128.41	102.73
513.3				9.50	0.2613	135.51	108.41
	0.375	12.85	1.50	13.00	0.3757	129.89	103.91
				12.75	0.3685	127.40	101.92
				13.25	0.3829	132.38	105.90
				12.75	0.3685	127.40	101.92
				13.25	0.3829	132.38	105.90
	0.500	17.00	2.00	17.50	0.5097	132.17	105.74
				17.00	0.4951	128.38	102.70
				17.25	0.5024	130.27	104.22
				17.50	0.5097	132.17	105.74
				16.75	0.4879	126.51	101.21
	0.750	25.50	3.00	25.50	0.7427	128.39	102.71
				26.00	0.7573	130.91	104.73
				26.25	0.7646	132.18	105.74
				25.25	0.7354	127.13	101.70
				25.75	0.7500	129.65	103.72
Average = 518.61 ± 24.33							Average = 103.96±2.10

Table XI

Albamycin T Tablet

Weight of preparation mg	Tetracycline HCl 99.03%		Pharmaceutical Preparation		Tetracycline HCl found mg	Tetracycline HCl found/ tablet mg	% Potency
	mg/10 ml	Average diffusion current	mg/10 ml	Diffusion current			
509.4 507.1	0.13	5.00	0.50	5.00 4.75 5.00 5.00 5.00	0.1287 0.1223 0.1287 0.1287 0.1287	130.82 124.32 130.82 130.82 130.82	104.66 99.46 104.66 104.66 104.66
Average = 508.25 ± 1.15	0.26	9.25	1.00	9.50 9.25 9.25 9.00 9.00	0.2644 0.2575 0.2575 0.2505 0.2505	134.38 130.87 130.87 127.32 127.32	107.50 104.70 104.70 101.86 101.86
	0.52	18.00	2.00	17.25 18.25 17.75 18.00 17.50	0.4935 0.5221 0.5078 0.5150 0.5007	125.41 132.68 129.04 130.87 127.24	100.33 106.14 103.23 104.70 101.77
	0.78	26.45	3.00	26.50 25.50 26.25 25.25 26.50	0.7739 0.7447 0.7666 0.7374 0.7739	131.11 126.17 129.88 124.93 131.11	104.89 100.94 103.90 99.94 104.89
	1.04	35.20	4.00	35.25 34.00 35.00 33.75 35.25	1.0314 0.9948 1.0241 0.9875 1.0314	131.05 126.40 130.12 125.47 131.05	104.89 101.12 104.10 100.38 104.84

Average = 103.39±2.14

Table XII

Albamycin T Granules

Weight of preparation gm	Tetracycline HCl 99.03%		Pharmaceutical Preparation		Tetracycline HCl found/ sample mg	Tetracycline HCl found/ vial mg	% Potency
	mg/10 ml	Average diffusion current	mg/10 ml	Diffusion current			
1.2605 1.3081	0.26	9.20	5.00	9.00 9.50 9.00 9.25 9.00	0.2519 0.2659 0.2519 0.2589 0.2519	64.70 68.30 64.70 66.50 64.70	103.52 109.28 103.52 106.40 103.52
Average = 1284.30 ± 8.2 mg	0.39	13.45	7.50	13.25 13.00 13.50 13.75 13.25	0.3805 0.3729 0.3876 0.3948 0.3805	65.16 63.86 66.37 67.61 65.16	104.26 102.18 106.19 108.18 104.26
	0.52	17.75	10.00	17.75 18.00 17.25 17.25 17.75	0.5150 0.5222 0.5005 0.5005 0.5150	66.14 67.07 64.28 64.28 66.14	105.82 107.31 102.85 102.85 105.82
	0.78	26.20	15.00	26.00 26.75 25.50 26.50 25.75	0.7665 0.7888 0.7518 0.7813 0.7592	65.63 67.54 64.37 66.89 65.00	105.01 108.06 102.99 107.02 104.00
	1.04	35.05	20.00	34.50 35.00 34.25 35.50 35.25	1.0137 1.0284 1.0064 1.0431 1.0358	65.09 66.04 64.63 66.98 66.51	104.14 105.66 103.41 107.17 106.42

Average = 105.19±1.95

Table XIII

Aureomycin Capsule

Weight of preparation mg	Cl-tetracycline HCl 98.49%		Pharmaceutical Preparation		Cl-tetracycline HCl found mg	Cl-tetracycline HCl found/capsule mg	% Potency
	mg/10 ml	Average diffusion current	mg/10 ml	Diffusion current			
				6.25	0.1638	258.74	103.50
				6.00	0.1573	248.47	99.39
411.1	0.165	6.20	0.25	6.25	0.1638	258.74	103.50
399.1				6.25	0.1638	258.74	103.50
403.8				6.25	0.1638	258.74	103.50
				12.00	0.3319	262.13	104.85
390.1				11.50	0.3183	251.24	100.50
396.6	0.330	11.75	0.50	12.00	0.3319	262.13	104.85
384.1				11.75	0.3250	256.68	102.67
				11.75	0.3250	256.68	102.67
393.1				17.00	0.4804	252.94	101.18
381.6				17.50	0.4946	260.42	104.17
407.6	0.495	17.25	0.75	18.00	0.5087	267.84	107.14
387.6				17.75	0.5016	264.11	105.64
				16.75	0.4734	249.21	99.70
403.1				23.25	0.6600	260.63	104.25
				23.00	0.6529	257.83	103.13
387.6	0.660	22.90	1.00	22.50	0.6387	252.22	100.89
388.6				23.00	0.6529	257.83	103.13
				23.75	0.6742	266.24	106.50
				34.25	0.9909	260.87	104.35
				34.50	0.9982	262.80	105.12
Average =	0.990	33.70	1.50	33.75	0.9765	257.08	102.83
394.90 ±				33.25	0.9620	253.27	101.31
8.93				34.00	0.9837	258.98	103.59

Average = 103.27±1.97

Table XIV

Declomycin Capsule

Weight of preparation mg	DMCT HCl 98.12%		Pharmaceutical Preparation		DMCT HCl found mg	DMCT HCl found/ capsule mg	% Potency
	mg/10 ml	Average diffusion current	mg/10 ml	Diffusion current			
339.3	0.230	7.60	0.50	7.75	0.2301	158.95	105.97
				8.00	0.2376	164.13	109.42
				7.50	0.2227	153.84	102.56
341.5				7.50	0.2227	153.84	102.56
				7.75	0.2301	158.95	105.97
340.1	0.345	11.00	0.75	11.00	0.3385	155.89	103.93
339.0				11.00	0.3385	155.89	103.93
351.4				11.50	0.3539	162.98	108.65
				11.25	0.3462	159.44	106.29
354.0				11.25	0.3462	159.44	106.29
350.0	0.460	14.25	1.00	14.25	0.4515	155.95	103.97
345.1				14.75	0.4672	161.37	107.58
				14.50	0.4593	158.64	105.76
346.1				14.00	0.4434	153.15	102.10
347.5				14.25	0.4515	155.95	103.97
Average = 345.40 ± 5.35	0.690	21.05	1.50	21.75	0.6995	161.07	107.38
				21.25	0.6835	157.39	104.93
				21.00	0.6754	155.52	103.68
				21.50	0.6915	159.23	106.15
				20.75	0.6674	153.70	102.47
	0.920	27.75	2.00	27.50	0.8946	154.50	103.00
				28.00	0.9108	157.30	104.87
				27.25	0.8864	153.08	102.05
				28.75	0.9352	161.51	107.67
				28.75	0.9352	161.51	107.67

Average = 105.15±2.16

Table XV

Declomycin Syrup

Volume of preparation	DMCT HCl 98.12%		Pharmaceutical Preparation		DMCT HCl found	DMCT HCl found/ml	% Potency
	mg/10 ml	Average diffusion current	ml/10 ml	Diffusion current	mg	mg	
2 fl.oz. bottles	0.20	6.50	0.0125	6.50	0.1962	15.70	104.67
				6.75	0.2038	16.30	108.67
				6.50	0.1962	15.70	104.67
				6.25	0.1886	15.09	100.60
				6.50	0.1962	15.70	104.67
	0.40	12.50	0.0250	12.50	0.3925	15.70	104.67
				12.25	0.3846	15.38	102.53
				12.75	0.4003	16.01	106.73
				12.50	0.3925	15.70	104.67
				12.75	0.4003	16.01	106.01
	0.60	18.50	0.0375	19.00	0.6046	16.12	107.47
				18.50	0.5887	15.70	104.67
				18.25	0.5808	15.49	103.27
				18.25	0.5808	15.49	103.27
				18.00	0.5728	15.27	101.80
	0.80	24.00	0.0500	23.75	0.7768	15.54	103.60
				24.00	0.7849	15.70	104.67
				24.75	0.8095	16.19	107.93
				23.50	0.7686	15.37	102.47
				24.25	0.7931	15.87	105.78
	1.00	29.70	0.0625	29.75	0.9828	15.72	104.80
				29.00	0.9581	15.32	102.13
				30.50	1.0076	16.12	107.47
				30.75	1.0159	16.25	108.33
				29.00	0.9581	15.32	102.13

Average = 104.71±2.19

Table XVI

Declostatin Capsule

Weight of preparation mg	DMCT HCl 98.12%		Pharmaceutical Preparation		DMCT HCl found mg	DMCT HCl found/ capsule mg	% Potency
	mg/10 ml	Average diffusion current	mg/10 ml	Diffusion current			
275.3	0.145	4.90	0.25	5.00	0.1452	160.37	106.91
				5.00	0.1452	160.37	106.91
280.7				4.75	0.1379	152.31	101.54
				5.00	0.1452	160.37	106.91
				5.00	0.1452	160.37	106.91
275.5	0.290	9.20	0.50	9.25	0.2861	158.00	105.33
274.9				9.50	0.2938	162.25	108.17
276.0				9.25	0.2861	158.00	105.33
				9.25	0.2861	158.00	105.33
277.0				9.00	0.2783	153.69	102.46
274.2	0.580	17.70	1.00	17.50	0.5627	155.37	103.58
				18.00	0.5787	159.79	106.53
276.9				17.25	0.5546	153.14	102.09
276.0				17.75	0.5707	157.58	105.05
274.7				18.25	0.5868	162.03	108.02
Average = 276.12 ± 1.84	0.870	26.25	1.50	25.75	0.8374	154.15	102.77
				26.75	0.8699	160.13	106.75
				26.00	0.8455	155.64	103.76
				26.75	0.8699	160.13	106.75
				26.50	0.8618	158.64	105.76
	1.160	35.75	2.00	36.00	1.1462	158.24	105.49
				36.75	1.1700	161.53	107.69
				35.00	1.1143	153.84	102.56
				35.50	1.1302	156.04	104.03
				35.25	1.1223	154.94	103.29

Average = 105.20±1.99

Table XVII

Terramycin Capsule

Weight of preparation mg	Oxytetracycline HCl 98.17%		Pharmaceutical Preparation		Oxytetracycline HCl found mg	Oxytetracycline found mg	Oxytetracycline found/capsule mg	% Potency
	mg/10 ml	Average diffusion current	mg/10 ml	Diffusion current				
				6.00	0.1883	0.1745	248.02	99.21
				6.25	0.1961	0.1817	258.26	103.30
733.5	0.195	6.10	0.50	6.00	0.1883	0.1745	248.02	99.21
741.4				6.25	0.1961	0.1817	258.26	103.30
				6.00	0.1883	0.1745	248.02	99.21
722.2				11.25	0.3745	0.3471	246.67	98.67
704.0				11.50	0.3829	0.3549	252.22	100.89
684.4	0.390	11.50	1.00	11.25	0.3745	0.3471	246.67	98.67
692.8				11.75	0.3912	0.3626	257.69	103.08
				12.00	0.3995	0.3703	263.16	105.26
706.7				17.00	0.5643	0.5230	247.79	99.12
717.7				17.25	0.5726	0.5307	251.44	100.58
	0.585	17.30	1.50	18.00	0.5975	0.5538	262.38	104.95
704.0				17.50	0.5809	0.5384	255.08	102.03
700.0				17.00	0.5643	0.5230	247.79	99.12
				23.00	0.7793	0.7223	256.66	102.66
				22.25	0.7539	0.6987	248.28	99.31
Average =	0.780	22.60	2.00	22.50	0.7623	0.7065	251.05	100.42
710.67±				23.25	0.7877	0.7300	259.40	103.76
17.85				22.00	0.7454	0.6908	245.49	98.20
				28.25	0.9487	0.8793	249.96	99.98
				28.00	0.9404	0.8716	247.77	99.11
	0.975	28.50	2.50	29.25	0.9823	0.9104	258.80	103.52
				28.75	0.9655	0.8948	254.36	101.74
				27.75	0.9320	0.8638	245.56	98.22

Average = 100.94±2.31

Table XVIII

Terramycin I.V. Powder

Weight of preparation	Oxytetracycline HCl 98.17%		Pharmaceutical Preparation		Oxytetracycline HCl found	Oxytetracycline HCl found/vial	% Potency
	mg/10 ml	Average diffusion current	mg/10 ml	Diffusion current	mg		
1.4463 gm	0.22	6.20	1.25	6.00	0.2090	241.81	96.72
				6.25	0.2177	251.88	100.75
				6.25	0.2177	251.88	100.75
				6.25	0.2177	251.88	100.75
				6.25	0.2177	251.88	100.75
	0.44	12.50	2.50	12.50	0.4320	249.92	99.97
				12.25	0.4233	244.89	97.96
				12.75	0.4406	254.90	101.96
				12.25	0.4233	244.89	97.96
				12.75	0.4406	254.90	101.96
	0.66	18.70	3.75	19.00	0.6583	253.89	101.56
				18.25	0.6323	243.86	97.54
				18.50	0.6410	247.22	98.89
				18.25	0.6323	243.86	97.54
				18.75	0.6496	250.54	100.22
	0.88	24.90	5.00	24.25	0.8414	243.38	97.55
				25.00	0.8674	250.90	100.36
				24.50	0.8500	245.87	98.35
				25.50	0.8847	255.91	102.36
				24.75	0.8587	248.39	99.36
	1.32	37.00	7.50	36.25	1.2696	244.83	97.93
				37.00	1.2958	249.88	99.95
				36.75	1.2871	248.20	99.28
				37.75	1.3221	254.95	101.98
				36.00	1.2608	243.13	97.25

Average = 99.59±1.73

Table XIX

Terramycin I.M. Solution

Volume of preparation	Oxytetracycline HCl 98.17%		Pharmaceutical Preparation		Oxytetracycline HCl found mg	Oxytetracycline HCl found/ ampoule mg	% Potency
	mg/10 ml	Average diffusion current	ml/10 ml	Diffusion current			
2 ml ampoules	0.510	14.25	0.010	14.75	0.5182	103.63	103.63
				14.25	0.5007	100.14	100.14
				14.50	0.5094	101.88	101.88
				14.00	0.4919	98.38	98.38
				14.00	0.4919	98.38	98.38
	0.765	22.10	0.015	22.00	0.7476	99.68	99.68
				22.75	0.7731	103.08	103.08
				22.25	0.7561	100.81	100.81
				21.75	0.7391	98.54	98.54
				22.00	0.7476	99.68	99.68
	1.020	29.00	0.020	29.00	1.0013	100.13	100.13
				29.75	1.0272	102.72	102.72
				28.00	0.9668	96.68	96.68
				28.75	0.9927	99.27	99.27
				28.25	0.9754	97.54	97.54
	1.275	36.50	0.025	36.50	1.2517	100.14	100.14
				35.50	1.2174	97.39	97.39
				36.25	1.2431	99.45	99.45
				37.25	1.2774	102.19	102.19
				35.75	1.2259	98.07	98.07
	1.530	43.95	0.030	42.75	1.4610	97.40	97.40
				44.50	1.5208	101.39	101.39
				44.00	1.5037	100.25	100.25
				43.75	1.4952	99.68	99.68
				45.00	1.5379	102.53	102.53

Average = 99.96±1.94

Table XX

Terramycin Otic

Weight of preparation mg	Oxytetracycline HCl 98.17%		Pharmaceutical Preparation		Oxytetracycline HCl found mg	Equivalent oxytetracycline base found mg	Oxytetracycline found/vial mg	% Potency
	mg/10 ml	Average diffusion current	mg/10 ml	Diffusion current				
272.00 mg	0.20	5.75	2.00	5.75	0.1963	0.1819	24.74	98.96
				5.75	0.1963	0.1819	24.74	98.96
				6.00	0.2049	0.1899	25.83	103.32
				5.50	0.1878	0.1740	23.66	94.64
				5.75	0.1963	0.1819	24.74	98.96
	0.40	11.30	4.00	11.50	0.3996	0.3703	25.18	100.72
				11.00	0.3822	0.3542	24.09	96.36
				11.25	0.3909	0.3623	24.64	98.56
				11.00	0.3822	0.3542	24.09	96.36
				11.50	0.3996	0.3703	25.18	100.72
	0.60	17.25	6.00	17.25	0.5890	0.5459	24.75	99.00
				17.00	0.5805	0.5380	24.39	97.56
				17.00	0.5805	0.5380	24.39	97.56
				17.50	0.5976	0.5538	25.10	100.40
				17.75	0.6061	0.5617	25.46	101.84
	0.80	23.00	8.00	23.25	0.7939	0.7358	25.02	100.08
				22.75	0.7768	0.7199	24.48	97.92
				23.00	0.7854	0.7279	24.75	99.00
				22.50	0.7683	0.7120	24.21	96.84
				22.75	0.7768	0.7199	24.48	97.92
	1.00	28.00	10.00	28.50	0.9992	0.9260	25.19	100.76
				27.25	0.9554	0.8854	24.08	96.32
				28.25	0.9905	0.9180	24.97	99.88
				27.75	0.9729	0.9017	24.53	98.12
				28.00	0.9817	0.9098	24.75	99.00

Average = 98.79±1.94

Table XXI

Terramycin Vaginal Tablet

Weight of preparation gm	Oxytetracycline HCl 98.17%		Pharmaceutical Preparation		Oxytetracycline HCl found mg	Equivalent oxytetracycline base found mg	Oxytetracycline found/tablet mg	% Potency
	mg/10 ml	Average diffusion current	mg/10 ml	Diffusion current				
1.2963	0.10	3.00	1.25	3.00	0.0982	0.0910	95.90	95.90
				2.875	0.0941	0.0872	91.91	91.91
				3.00	0.0982	0.0910	95.90	95.90
1.2865				2.875	0.0941	0.0872	91.91	91.91
				3.00	0.0982	0.0910	95.90	95.90
1.2854	0.20	5.80	2.50	6.00	0.2031	0.1882	99.21	99.21
1.3768				5.50	0.1862	0.1726	90.97	90.97
1.2962				5.75	0.1946	0.1804	95.08	95.08
				5.75	0.1946	0.1804	95.08	95.08
1.2929				5.75	0.1946	0.1804	95.08	95.08
1.3766	0.40	11.25	5.00	11.50	0.4014	0.3720	98.03	98.03
				11.00	0.3840	0.3559	93.79	93.79
1.3195				10.75	0.3752	0.3477	91.63	91.63
1.3133				11.25	0.3953	0.3664	96.56	96.56
1.3330				11.25	0.3953	0.3664	96.56	96.56
Average = 1317.65 mg ± 32.82	0.60	17.00	7.50	17.00	0.5890	0.5459	95.91	95.91
				16.75	0.5803	0.5378	94.49	94.49
				17.25	0.5977	0.5539	97.35	97.35
				17.00	0.5890	0.5459	95.91	95.91
				16.50	0.5717	0.5298	93.08	93.08
	0.80	22.95	10.00	22.75	0.7785	0.7215	95.06	95.06
				22.25	0.7614	0.7056	92.97	92.97
				22.50	0.7700	0.7136	94.02	94.02
				23.50	0.8042	0.7453	98.20	98.20
				23.00	0.7871	0.7295	96.12	96.12

Average = 95.04±2.10

Table XXII

Terramycin Pediatric Drops

Volume of preparation	Ca-diterramycin 87.13%		Pharmaceutical Preparation		Ca-diterramycin found	Equivalent oxytetracycline found	Oxytetracycline found/ml	% Potency
	mg/10 ml	Average diffusion current	ml/10 ml	Diffusion current	mg	mg	mg	
10 ml bottles	0.150	4.60	0.00125	4.50	0.1278	0.1225	98.00	98.00
				4.75	0.1350	0.1294	103.52	103.52
				4.50	0.1278	0.1225	98.00	98.00
				4.50	0.1278	0.1225	98.00	98.00
				4.75	0.1350	0.1294	103.52	103.52
	0.300	8.55	0.0025	8.75	0.2675	0.2564	102.56	102.56
				8.50	0.2599	0.2491	99.64	99.64
				8.75	0.2675	0.2564	102.56	102.56
				8.50	0.2599	0.2491	99.64	99.64
				8.50	0.2599	0.2491	99.64	99.64
	0.450	12.50	0.00375	12.50	0.3921	0.3758	100.21	100.21
				13.00	0.4078	0.3908	104.21	104.21
				12.75	0.3999	0.3833	102.21	102.21
				12.25	0.3842	0.3682	98.19	98.19
				12.50	0.3921	0.3758	100.21	100.21
	0.600	16.50	0.0050	17.00	0.5386	0.5162	103.24	103.24
				16.75	0.5307	0.5086	101.72	101.72
				16.50	0.5228	0.5010	100.20	100.20
				16.25	0.5149	0.4935	98.70	98.70
				16.50	0.5228	0.5010	100.20	100.20
	0.900	24.20	0.0075	24.00	0.7777	0.7453	99.37	99.37
				25.00	0.8101	0.7764	103.52	103.52
				24.50	0.7939	0.7609	101.45	101.45
				23.75	0.7696	0.7376	98.34	98.34
				24.75	0.8020	0.7686	102.48	102.48

Average = 100.78±2.04

Table XXIII

Chloromycetin Ophthalmic

Weight of preparation mg	Chloramphenicol		Pharmaceutical Preparation		Chloramphenicol found mg	Chloramphenicol found/vial mg	% Potency
	mg/10 ml	Average diffusion current	mg/10 ml	Diffusion current			
126.50 mg	0.20	16.30	1.00	16.00	0.1963	24.83	99.32
				16.50	0.2024	25.60	102.40
				16.25	0.1994	25.22	100.88
				16.75	0.2055	26.00	104.00
				16.50	0.2024	25.60	102.40
	0.60	49.00	3.00	49.00	0.6000	25.30	101.20
				50.00	0.6122	25.81	103.24
				48.00	0.5878	24.79	99.16
				49.50	0.6061	25.56	102.24
				48.75	0.5969	25.17	100.68
	0.80	65.05	4.00	64.00	0.7871	24.89	99.56
				65.00	0.7994	25.28	101.12
				66.25	0.8148	25.77	103.08
				64.50	0.7932	25.08	100.32
				66.50	0.8178	25.86	103.44
	1.00	80.50	5.00	82.00	1.0186	25.77	103.08
				78.50	0.9752	24.67	98.68
				81.00	1.0062	25.46	101.81
				80.50	1.0000	25.30	101.20
				82.50	1.0248	25.93	103.72
	0.40	32.50	2.00	32.50	0.4000	25.30	101.20
				32.00	0.3938	24.91	99.64
				33.25	0.4092	25.88	103.52
				31.50	0.3877	24.52	98.08
				33.00	0.4062	25.69	102.96

Average = 101.47±1.72

Table XXIV

Erythrocin Chewable Tablet

Weight of preparation mg	Erythromycin Ethyl Succinate		Pharmaceutical Preparation		Erythromycin Ethyl Succinate found mg	Equivalent Erythromycin mg	Erythromycin found/ tablet mg	% Potency
	mg/10 ml	Average diffusion current	mg/10 ml	Diffusion current				
				12.00	0.0871	0.0705	209.14	104.57
				12.25	0.0890	0.0720	213.60	106.80
746.9	0.0875	12.05	0.25	11.75	0.0853	0.0690	204.70	102.35
749.6				12.25	0.0890	0.0720	213.60	106.80
				12.00	0.0871	0.0705	209.14	104.57
743.3				22.50	0.1697	0.1373	203.66	101.83
732.4				23.75	0.1791	0.1449	214.93	107.46
743.5	0.1750	23.20	0.50	23.25	0.1754	0.1419	210.48	105.24
740.8				23.00	0.1734	0.1404	208.26	104.13
				23.25	0.1754	0.1419	210.48	105.24
730.4				34.75	0.2683	0.2171	214.69	107.34
751.4				34.00	0.2625	0.2124	210.04	105.02
742.6	0.2625	34.00	0.75	33.50	0.2586	0.2093	206.98	103.49
735.7				34.50	0.2664	0.2156	213.21	106.60
				33.25	0.2567	0.2077	205.39	102.70
				45.00	0.3500	0.2832	210.04	105.02
				44.00	0.3422	0.2769	205.37	102.68
Average =	0.3500	45.00	1.00	44.25	0.3442	0.2786	206.63	103.32
741.66 ±				45.75	0.3558	0.2879	213.52	106.76
7.00				45.75	0.3558	0.2879	213.52	106.76
				64.00	0.5106	0.4132	204.30	102.15
				67.00	0.5346	0.4326	213.89	106.94
	0.5250	65.80	1.50	65.00	0.5186	0.4197	207.53	103.76
				66.00	0.5266	0.4262	210.73	105.36
				67.50	0.5386	0.4359	215.53	107.76

Average = 104.99±1.85

Table XXV

Erythrocin Granules

Weight of preparation gm	Erythromycin Ethyl Succinate		Pharmaceutical Preparation		Erythromycin Ethyl Succinate found mg	Erythromycin Ethyl Succinate found/ vial gm	% Potency
	mg/10 ml	Average diffusion current	mg/10 ml	Diffusion current			
21.1993	0.059	8.55	0.50	8.50	0.0587	2.4888	103.70
				8.50	0.0587	2.4888	103.70
				8.75	0.0604	2.5609	106.70
				8.50	0.0587	2.4888	103.70
				8.75	0.0604	2.5609	106.70
	0.118	15.75	1.00	16.00	0.1199	2.5418	105.91
				15.75	0.1180	2.5015	104.38
				16.25	0.1218	2.5820	107.58
				15.50	0.1161	2.4612	102.55
				15.50	0.1161	2.4612	102.55
	0.177	23.10	1.50	23.50	0.1801	2.5454	106.06
				23.00	0.1762	2.4902	102.76
				23.25	0.1781	2.5171	104.88
				22.50	0.1724	2.4365	101.52
				23.25	0.1781	2.5171	104.88
	0.295	37.75	2.50	37.50	0.2930	2.4846	103.53
				38.75	0.3028	2.5676	106.98
				37.00	0.2891	2.4515	102.15
				38.00	0.2970	2.5185	104.94
				37.75	0.2950	2.5015	104.23
	0.413	52.00	3.50	51.00	0.4051	2.4536	102.23
				51.00	0.4051	2.4536	102.23
				51.50	0.4090	2.4773	103.22
				53.25	0.4229	2.5615	106.73
				52.50	0.4170	2.5257	105.24

Average = 104.40±1.75

Table XXVI

Erythrocin I.M.

Volume of preparation ml	Erythromycin Ethyl Succinate		Pharmaceutical Preparation		Erythromycin Ethyl Succinate found mg	Equivalent Erythromycin mg	Erythromycin found/ ampoule mg	% Potency
	mg/10 ml	Average diffusion current	ml/10 ml	Diffusion current				
2 ml ampoules	0.125	16.50	0.002	16.75	0.1269	0.1027	102.70	102.70
				16.25	0.1231	0.0996	99.60	99.60
				16.50	0.1250	0.1012	101.20	101.20
				16.00	0.1212	0.0981	98.10	98.10
				16.25	0.1231	0.0996	99.60	99.60
	0.250	32.00	0.004	31.75	0.2480	0.2007	100.35	100.35
				31.00	0.2422	0.1960	98.00	98.00
				32.50	0.2539	0.2055	102.75	102.75
				31.00	0.2422	0.1960	98.00	98.00
				32.75	0.2559	0.2071	103.55	103.55
	0.375	47.30	0.006	47.25	0.3746	0.3031	101.03	101.03
				46.00	0.3647	0.2951	98.37	98.37
				48.25	0.3825	0.3095	103.17	103.17
				46.50	0.3686	0.2983	99.43	99.43
				46.00	0.3647	0.2951	98.37	98.37
	0.500	62.70	0.008	63.00	0.5024	0.4066	101.65	101.65
				60.75	0.4844	0.3920	98.00	98.00
				63.75	0.5084	0.4114	102.85	102.85
				62.25	0.4964	0.4017	100.42	100.42
				62.00	0.4944	0.4001	100.02	100.02
	0.625	78.00	0.010	77.00	0.6170	0.4993	99.86	99.86
				75.50	0.6050	0.4896	97.92	97.92
				78.75	0.6310	0.5106	102.12	102.12
				79.50	0.6370	0.5155	103.10	103.10
				76.75	0.6150	0.4977	99.54	99.54

Average = 100.39±1.88

Table XXVII

Olicin

Weight of preparation mg	Triacetyl Oleandomycin 98.70%		Pharmaceutical Preparation		Triacetyl Oleando-mycin found mg	Equivalent oleando-mycin found mg	Oleando-mycin found/ capsule mg	% Potency
	mg/10 ml	Average diffusion current	mg/10 ml	Diffusion current				
				12.00	0.0561	0.0476	247.01	98.80
				12.25	0.0572	0.0485	251.68	100.67
643.5	0.0580	12.25	0.125	12.50	0.0584	0.0495	256.86	102.74
635.8				12.00	0.0561	0.0476	247.01	98.80
				12.25	0.0572	0.0485	251.68	100.67
645.9				24.50	0.1147	0.0973	252.45	100.98
641.6				25.00	0.1171	0.0993	257.64	103.06
657.9	0.1160	24.45	0.250	24.25	0.1136	0.0963	249.86	99.94
651.5				24.00	0.1124	0.0953	247.26	98.90
				24.00	0.1124	0.0953	247.26	98.90
671.7				35.50	0.1659	0.1407	243.37	97.35
651.5				36.00	0.1682	0.1426	246.66	98.66
	0.1740	36.75	0.375	37.25	0.1741	0.1476	255.30	102.12
634.3				36.75	0.1717	0.1456	251.84	100.74
652.8				35.50	0.1659	0.1407	243.37	97.35
				48.00	0.2262	0.1918	248.82	99.53
				47.25	0.2226	0.1888	244.93	97.97
Average =	0.2320	48.60	0.500	49.75	0.2344	0.1988	257.90	103.16
648.65 ±				47.50	0.2238	0.1898	246.23	98.49
11.85				49.25	0.2320	0.1967	255.18	102.07
				73.75	0.3414	0.2895	250.38	100.15
				72.50	0.3356	0.2846	246.14	98.46
	0.3480	74.20	0.750	76.00	0.3518	0.2983	257.99	103.20
				74.50	0.3449	0.2925	252.97	101.19
				73.75	0.3414	0.2895	250.38	100.15

Average = 100.16±1.82

Appendix II

Polarographic determination of the recovery of the antibiotics in pharmaceutical preparations.

In each case the pure antibiotic was added to give the theoretical amount present. The amount found and the amount recovered were then determined and the recovery calculated as a percentage.

Table I

Tetracyn Capsule

Amount of preparation mg/10 ml	Tetracycline HCl (99.03%)							
	Amount added mg	Total amount present mg/10 ml	Diffusion current	Amount of pure mg/10 ml	Average diffusion current	Total amount found mg/10 ml	Amount recovered mg	% Recovery
0.75 ≡ 250x0.75x1.0717 / 674.29 = 0.2974 mg Tetracycline HCl	0.125	0.4253	14.50 14.50 14.75 14.50 14.50	0.425	14.60	0.4221 0.4221 0.4294 0.4221 0.4221	0.1218 0.1218 0.1291 0.1218 0.1218	97.44 97.44 103.28 97.44 97.44
	0.250	0.5503	18.75 18.50 18.50 18.25 18.50	0.550	18.50	0.5574 0.5500 0.5500 0.5421 0.5500	0.2571 0.2497 0.2497 0.2423 0.2497	102.84 99.88 99.88 96.92 99.88
≡0.3003 mg Tetracycline HCl (as 99.03%)	0.375	0.6753	22.75 23.25 22.75 23.00 22.75	0.675	23.00	0.6677 0.6824 0.6677 0.6750 0.6677	0.3674 0.3788 0.3674 0.3747 0.3674	97.97 101.01 97.97 99.92 97.97
	0.500	0.8003	27.00 27.00 27.50 26.75 27.75	0.800	27.25	0.7927 0.7927 0.8073 0.7853 0.8146	0.4924 0.4924 0.5070 0.4850 0.5116	98.48 98.48 101.40 97.00 102.32
	0.750	1.0503	35.50 35.00 34.50 35.00 34.75	1.050	35.10	1.0619 1.0470 1.0320 1.0470 1.0395	0.7616 0.7467 0.7317 0.7467 0.7392	101.54 99.56 97.56 99.56 98.56

Average = 99.51±

Table II

Signemycin Capsule

Amount of preparation mg/10 ml	Tetracycline HCl (99.03%)							
	Amount added mg	Total amount present mg/10 ml	Diffusion current	Amount of pure mg/10 ml	Average diffusion current	Total amount found mg/10 ml	Amount recovered mg	% Recovery
0.625 mg ≡ $\frac{167 \times 0.625 \times 1.0443}{403.04}$ =0.2705 mg Tetracycline HCl	0.125	0.3982	14.25 14.25 14.50 14.25 14.25	0.3980	14.25	0.3980 0.3980 0.4050 0.3980 0.3980	0.1248 0.1248 0.1318 0.1248 0.1248	99.84 99.84 105.44 99.84 99.84
	0.250	0.5232	18.75 18.50 18.75 18.25 18.50	0.5230	18.50	0.5301 0.5230 0.5301 0.5159 0.5230	0.2569 0.2498 0.2569 0.2427 0.2498	102.76 99.92 102.76 97.08 99.92
≡0.2732 mg Tetracycline HCl (as 99.03%)	0.375	0.6482	22.00 22.25 22.25 21.75 22.00	0.6480	22.00	0.6480 0.6554 0.6554 0.6406 0.6480	0.3748 0.3822 0.3822 0.3674 0.3748	99.95 101.92 101.92 97.97 99.95
	0.50	0.7732	26.25 26.75 27.25 26.50 27.00	0.7730	26.70	0.7599 0.7744 0.7889 0.7672 0.7816	0.4867 0.5012 0.5157 0.4940 0.5084	97.34 100.24 102.14 98.80 101.68
	0.75	1.0232	35.00 35.50 34.25 34.75 35.50	1.0230	34.90	1.0258 1.0405 1.0039 1.0185 1.0405	0.7526 0.7673 0.7307 0.7453 0.7673	100.34 102.3 97.42 99.37 102.3

Table III

Tetrosol

Amount of preparation mg/10 ml	Tetracycline HCl (99.03%)							
	Amount added mg	Total amount present mg/10 ml	Diffusion current	Amount of pure mg/10 ml	Average diffusion current	Total amount found mg/10 ml	Amount recovered mg	% Recovery
0.4 mg ≡ 250x0.4x0.9748 / 519.16 ≡0.1878 mg Tetracycline HCl ≡0.1896 mg Tetracycline HCl (as 99.03%)	0.125	0.3146	11.50 11.50 11.625 11.375 11.50	0.315	11.50	0.3150 0.3150 0.3184 0.3116 0.3150	0.1254 0.1254 0.1288 0.1220 0.1254	100.32 100.32 103.04 97.60 100.32
	0.250	0.4396	15.25 15.50 15.50 15.25 15.25	0.440	15.30	0.4386 0.4458 0.4458 0.4386 0.4386	0.2490 0.2562 0.2562 0.2490 0.2490	99.60 102.48 102.48 99.60 99.60
	0.375	0.5646	19.25 20.00 19.50 19.25 19.75	0.565	19.50	0.5577 0.5794 0.5650 0.5577 0.5721	0.3681 0.3898 0.3754 0.3681 0.3825	98.17 103.96 100.12 98.17 102.01
	0.500	0.6896	24.50 24.00 23.75 24.25 24.25	0.690	24.00	0.7044 0.6900 0.6828 0.6972 0.6972	0.5148 0.5004 0.4932 0.5076 0.5076	102.96 100.08 98.64 101.52 101.52
	0.750	0.9396	32.25 32.75 31.50 32.00 32.50	0.940	32.10	0.9443 0.9589 0.9223 0.9370 0.9516	0.7547 0.7693 0.7327 0.7474 0.7620	100.60 102.55 97.67 99.63 101.57

Table IV

Achromycin Capsule

Amount of preparation mg/10 ml	Tetracycline HCl (99.03%							
	Amount added mg	Total amount present mg/10 ml	Diffusion current	Amount of pure mg/10 ml	Average diffusion current	Total amount found mg/10 ml	Amount recovered mg	% Recovery
0.4 mg =0.4x250x1.013 / 386.14 =0.2623 mg Tetracycline HCl	0.125	0.3899	13.50 13.375 13.50 13.50 13.375	0.3900	13.50	0.3900 0.3864 0.3900 0.3900 0.3864	0.1251 0.1215 0.1251 0.1251 0.1215	100.08 97.20 100.08 100.08 97.20
	0.250	0.5149	18.00 18.25 17.75 17.75 18.00	0.5150	18.00	0.5150 0.5220 0.5076 0.5076 0.5150	0.2501 0.2571 0.2427 0.2427 0.2501	100.04 102.84 97.08 97.08 100.04
=0.2649 mg Tetracycline HCl (as 99.03%)	0.375	0.6399	22.00 21.50 21.75 22.00 21.75	0.6400	21.80	0.6459 0.6312 0.6386 0.6459 0.6386	0.3810 0.3663 0.3737 0.3810 0.3737	101.61 97.69 99.66 101.61 99.66
	0.500	0.7649	26.00 25.50 26.50 25.75 26.25	0.7650	26.20	0.7592 0.7446 0.7738 0.7519 0.7665	0.4943 0.4797 0.5089 0.4870 0.5016	98.86 95.94 101.78 97.40 100.32
	0.750	1.0149	35.00 34.00 33.75 34.50 34.25	1.0150	34.45	1.0311 1.0016 0.9943 1.0164 1.0090	0.7662 0.7367 0.7294 0.7515 0.7441	102.13 98.20 97.23 100.17 99.19

Table V

Achromycin Syrup

Amount of preparation ml/10 ml	Tetracycline HCl (99.03%)							
	Amount added mg	Total amount present mg/10 ml	Diffusion current	Amount of pure mg/10 ml	Average diffusion current	Total amount found mg/10 ml	Amount recovered mg	% Recovery
0.01 ml $=\frac{25 \times 0.01 \times 0.9959}{1}$ =0.2490 mg Tetracycline HCl	0.125	0.3764	13.00 13.25 13.00 13.00 13.00	0.375	13.00	0.3744 0.3823 0.3744 0.3744 0.3744	0.1230 0.1309 0.1230 0.1230 0.1230	104.72 98.40 104.72 104.72 104.72
	0.250	0.5014	17.50 17.50 17.25 17.25 17.50	0.500	17.40	0.5030 0.5030 0.4958 0.4958 0.5030	0.2516 0.2516 0.2444 0.2444 0.2516	100.64 100.64 97.76 97.76 100.64
=0.2514 mg Tetracycline HCl (as 99.03%)	0.375	0.6264	21.00 21.50 21.25 21.00 21.25	0.625	21.25	0.6176 0.6323 0.6250 0.6176 0.6250	0.3662 0.3809 0.3736 0.3662 0.3736	97.67 101.59 99.64 97.67 99.64
	0.500	0.7514	25.75 26.25 25.50 25.75 25.50	0.750	25.75	0.7500 0.7647 0.7428 0.7500 0.7428	0.4986 0.5133 0.4914 0.4986 0.4914	99.72 102.66 98.28 99.72 98.28
	0.750	1.0014	34.25 34.00 33.25 33.75 33.50	1.000	33.90	1.0178 1.0030 0.9809 0.9956 0.9882	0.7664 0.7516 0.7295 0.7442 0.7368	102.16 100.19 97.24 99.20 98.22

Table VI

Achrocidin Tablet

Amount of preparation mg/10 ml	Tetracycline HCl (99.03%)							
	Amount added mg	Total amount present mg/10 ml	Diffusion current	Amount of pure mg/10 ml	Average diffusion current	Total amount found mg/10 ml	Amount recovered mg	% Recovery
1.5 mg $=\frac{125 \times 1.5 \times 1.0013}{783.45}$ =0.2420 mg Tetracycline HCl	0.125	0.3694	13.00 13.125 13.00 13.875 13.00	0.370	13.00	0.3700 0.3735 0.3700 0.3664 0.3700	0.1256 0.1291 0.1256 0.1220 0.1256	100.48 103.28 100.48 97.60 100.48
	0.250	0.4944	16.75 17.00 17.25 16.00 17.00	0.495	16.90	0.4906 0.4979 0.5052 0.4906 0.4979	0.2462 0.2535 0.2608 0.2462 0.2535	98.48 101.40 104.32 98.48 101.40
=0.2444 mg Tetracycline HCl (as 99.03%)	0.375	0.6194	21.50 21.25 21.00 21.25 21.50	0.620	21.25	0.6274 0.6200 0.6128 0.6200 0.6274	0.3830 0.3756 0.3684 0.3756 0.3830	102.15 100.17 98.25 100.17 102.15
	0.500	0.7444	24.75 25.50 25.00 24.75 25.50	0.745	25.15	0.7331 0.7553 0.7405 0.7331 0.7553	0.4887 0.5109 0.4961 0.4887 0.5109	97.74 102.18 99.22 97.74 102.18
	0.750	0.9944	33.00 33.50 33.00 34.25 34.00	0.995	33.50	0.9801 0.9950 0.9801 1.0172 1.0098	0.7357 0.7506 0.7357 0.7728 0.7654	98.07 100.05 98.07 103.01 102.03

Table VII

Achrocidin Compound Syrup

Amount of preparation ml/10 ml	Tetracycline HCl (99.03%)							
	Amount added mg	Total amount present mg/10 ml	Diffusion current	Amount of pure mg/10 ml	Average diffusion current	Total amount found mg/10 ml	Amount recovered mg	% Recovery
0.01 ml $=\frac{25 \times 0.01 \times 1.0474}{1}$ =0.2618 mg Tetracycline HCl =0.2644 mg Tetracycline HCl (as 99.03%)	0.125	0.3894	14.00 13.75 13.75 13.75 13.75	0.3900	13.80	0.3956 0.3886 0.3886 0.3886 0.3886	0.1312 0.1242 0.1242 0.1242 0.1242	104.96 99.36 99.36 99.36 99.36
	0.250	0.5144	18.00 17.75 17.50 17.75 17.50	0.515	17.75	0.5222 0.5150 0.5077 0.5135 0.5077	0.2578 0.2506 0.2433 0.2491 0.2433	103.12 100.24 97.32 99.64 97.32
	0.375	0.6394	22.00 22.50 22.00 22.50 22.25	0.640	22.30	0.6314 0.6458 0.6314 0.6458 0.6386	0.3670 0.3814 0.3670 0.3814 0.3742	97.88 101.72 97.88 101.72 99.80
	0.500	0.7644	26.25 26.75 25.75 26.50 26.00	0.765	26.25	0.7650 0.7795 0.7504 0.7722 0.7576	0.5006 0.5151 0.4860 0.5078 0.4932	100.12 103.02 97.20 101.56 98.64
	0.750	1.0144	34.75 34.25 35.25 34.25 33.75	1.015	34.60	1.0196 1.0049 1.0342 1.0049 0.9902	0.7552 0.7405 0.7698 0.7405 0.7258	100.67 98.71 102.61 98.71 96.75



Table VIII

Tetrex Capsule

Amount of preparation mg/10 ml	Tetracycline HCl (99.03%)							
	Amount Added mg	Total amount present mg/10 ml	Diffusion current	Amount of pure mg/10 ml	Average diffusion current	Total amount found mg/10 ml	Amount recovered mg	% Recovery
0.50 mg ≡ 100x0.5x1.06 / 248.14 = 0.2136 mg Tetracycline HCl	0.125	0.3407	11.75 11.50 11.50 11.75 11.50	0.340	11.60	0.3444 0.3371 0.3371 0.3444 0.3371	0.1287 0.1214 0.1214 0.1287 0.1214	102.96 97.12 97.12 102.96 97.12
	0.250	0.4657	15.75 16.00 16.00 16.00 16.25	0.465	16.00	0.4577 0.4650 0.4650 0.4650 0.4722	0.2420 0.2493 0.2493 0.2493 0.2565	96.80 99.72 99.72 99.72 102.60
≡0.2157 mg Tetracycline HCl (as 99.03%)	0.375	0.5907	20.25 19.75 20.00 19.75 20.00	0.590	20.00	0.5974 0.5826 0.5900 0.5826 0.5900	0.3817 0.3669 0.3773 0.3669 0.3743	101.80 97.85 99.83 97.85 99.83
	0.500	0.7157	24.00 23.50 24.50 23.75 24.00	0.715	24.00	0.7150 0.7001 0.7298 0.7075 0.7150	0.4993 0.4844 0.5141 0.4918 0.4993	99.86 96.88 102.82 98.36 99.86
	0.750	0.9657	32.00 32.25 32.00 32.75 32.50	0.965	32.30	0.9562 0.9636 0.9562 0.9786 0.9711	0.7405 0.7479 0.7405 0.7629 0.7554	98.71 99.70 98.71 101.69 100.69

Table IX

Tetrex I.M.

Amount of Preparation mg/10 ml	Tetracycline HCl (99.03%)							
	Amount added mg	Total amount present mg/10 ml	Diffusion current	Amount of pure mg/10 ml	Average diffusion current	Total amount found mg/10 ml	Amount recovered mg	% Recovery
1.0 mg ≡ 250x1x10323 / 897.50 =0.2875 mg Tetracycline HCl ≡0.2903 mg Tetracycline HCl (as 99.03%)	0.125	0.4153	14.25 14.25 14.00 14.25 14.00	0.415	14.15	0.4180 0.4180 0.4106 0.4180 0.4106	0.1277 0.1277 0.1203 0.1277 0.1203	102.16 102.16 96.24 102.16 96.24
	0.250	0.5403	18.25 18.50 18.75 18.50 18.75	0.540	18.50	0.5327 0.5400 0.5473 0.5400 0.5473	0.2424 0.2497 0.2570 0.2497 0.2570	96.96 99.88 102.80 99.88 102.80
	0.375	0.6653	22.75 22.50 22.75 22.50 23.00	0.665	22.70	0.6666 0.6592 0.6666 0.6592 0.6739	0.3763 0.3689 0.3763 0.3689 0.3836	100.36 98.39 100.36 98.39 102.31
	0.500	0.7903	26.75 27.50 27.00 27.25 27.00	0.790	27.00	0.7827 0.8046 0.7900 0.7973 0.7900	0.4924 0.5143 0.4997 0.5070 0.4997	98.48 102.86 99.94 101.40 99.94
	0.750	1.0403	36.00 35.00 35.50 36.00 35.00	1.040	35.40	1.0577 1.0283 1.0430 1.0577 1.0283	0.7674 0.7380 0.7527 0.7674 0.7380	102.29 98.38 100.33 102.29 98.38

Table X

Azotrex

Amount of preparation mg/10 ml	Tetracycline HCl (99.03%)							
	Amount added mg	Total amount present mg/10 ml	Diffusion current	Amount of pure mg/10 ml	Average diffusion current	Total amount found mg/10 ml	Amount recovered mg	% Recovery
1.0 mg ≡ 125x1x1.0396 / 518.61 =0.2506 mg Tetracycline HCl ≡0.2530 mg Tetracycline HCl (as 99.03%)	0.125	0.378	13.25 12.75 13.00 13.25 12.75	0.375	13.00	0.3822 0.3678 0.3750 0.3822 0.3678	0.1292 0.1226 0.1250 0.1292 0.1226	103.36 98.08 100.00 103.36 98.08
	0.250	0.503	17.50 17.75 17.25 17.50 17.25	0.500	17.45	0.5014 0.5086 0.4943 0.5014 0.4943	0.2484 0.2556 0.2413 0.2484 0.2413	99.36 102.24 96.52 99.36 96.52
	0.375	0.628	21.25 21.00 21.75 21.75 21.00	0.625	21.30	0.6235 0.6162 0.6382 0.6382 0.6162	0.3705 0.3632 0.3852 0.3852 0.3632	98.80 96.85 102.72 102.72 96.85
	0.500	0.753	25.50 26.00 25.75 25.25 25.00	0.750	25.50	0.7500 0.7647 0.7574 0.7426 0.7353	0.4970 0.5117 0.5044 0.4896 0.4823	99.40 102.34 100.88 97.92 96.46
	0.750	1.003	34.00 34.50 33.75 33.25 33.50	1.000	33.75	1.0074 1.0222 1.0000 0.9852 0.9926	0.7544 0.7692 0.7470 0.7322 0.7396	100.58 102.56 99.60 97.62 98.61

Table XI

Albamycin T Tablet

Amount of preparation mg/10 ml	Tetracycline HCl (99.03%)							
	Amount added mg	Total amount present mg/10 ml	Diffusion current	Amount of pure mg/10 ml	Average diffusion current	Total amount found mg/10 ml	Amount recovered mg	% Recovery
1.00 mg $=\frac{125x1x1.0339}{508.25}$ =0.2543 mg Tetracycline HCl $=$0.2568 mg Tetracycline HCl (as 99.03%)	0.125	0.3818	13.00 13.25 13.25 13.125 13.125	0.3812	13.15	0.3769 0.3841 0.3841 0.3805 0.3805	0.1201 0.1273 0.1273 0.1237 0.1237	96.08 101.84 101.84 98.96 98.96
	0.250	0.5068	17.75 17.25 17.75 17.50 17.50	0.5062	17.50	0.5135 0.4990 0.5135 0.5062 0.5062	0.2567 0.2422 0.2567 0.2494 0.2494	102.68 96.88 102.68 99.76 99.76
	0.375	0.6318	21.75 21.75 22.00 22.25 21.50	0.6312	21.90	0.6268 0.6268 0.6340 0.6412 0.6194	0.3700 0.3700 0.3772 0.3844 0.3626	98.68 98.68 100.60 102.52 96.70
	0.500	0.7568	25.25 25.50 25.25 26.00 25.50	0.7562	25.60	0.7459 0.7533 0.7459 0.7680 0.7533	0.4891 0.4965 0.4891 0.5112 0.4965	97.82 99.30 97.82 102.24 99.30
	0.750	1.0068	34.50 33.50 34.00 33.50 33.75	1.0062	34.00	1.0208 0.9912 1.0062 0.9912 0.9987	0.7640 0.7344 0.7494 0.7344 0.7419	101.84 97.90 99.90 97.90 98.90

Table XII

Albamycin T Granules

Amount of preparation mg/10 ml	Tetracycline HCl (99.03%)							
	Amount added mg	Total amount present mg/10 ml	Diffusion current	Amount of pure mg/10 ml	Average diffusion current	Total amount found mg/10 ml	Amount recovered mg	% Recovery
4.00 =4x62.5x1.0519 1284.30 =0.2048 mg Tetracycline HCl =0.2068 mg Tetracycline HCl (as 99.03%)	0.125	0.3318	11.625 11.625 11.50 11.50 11.50	0.3312	11.50	0.3362 0.3362 0.3312 0.3312 0.3312	0.1294 0.1294 0.1244 0.1244 0.1244	103.52 103.52 99.52 99.52 99.52
	0.250	0.4568	16.00 16.25 15.75 15.875 16.125	0.4562	15.95	0.4576 0.4648 0.4504 0.4540 0.4612	0.2508 0.2580 0.2436 0.2472 0.2544	100.32 103.20 97.44 98.88 101.76
	0.375	0.5818	19.50 19.50 20.25 19.75 20.00	0..5812	19.75	0.5739 0.5739 0.5960 0.5812 0.5886	0.3671 0.3671 0.3892 0.3744 0.3818	97.91 97.91 103.80 99.85 101.83
	0.500	0.7068	24.25 23.75 24.00 24.50 24.00	0.7062	24.00	0.7134 0.6987 0.7062 0.7208 0.7062	0.5066 0.4919 0.4994 0.5140 0.4994	101.32 98.38 99.88 102.80 99.88
	0.750	0.9568	28.25 27.75 28.00 28.75 28.25	0.9562	28.15	0.9596 0.9427 0.9511 0.9766 0.9596	0.7528 0.7359 0.7443 0.7698 0.7528	100.35 98.10 99.22 102.61 100.35

Table XIII

Aureomycin Capsule

Amount of preparation mg/10 ml	Chlorotetracycline HCl (98.49%)							
	Amount added mg	Total amount present mg/10 ml	Diffusion current	Amount of pure mg/10 ml	Average diffusion current	Total amount found mg/10 ml	Amount recovered mg	% Recovery
0.40 mg $=\frac{250 \times 0.4 \times 1.0327}{394.90}$ =0.2615 Chlorotetracycline HCl ≡0.2655 mg Chlorotetracycline HCl (as 98.49%)	0.125	0.3905	11.375 11.50 11.50 11.375 11.50	0.3900	11.45	0.3874 0.3917 0.3917 0.3874 0.3917	0.1219 0.1262 0.1262 0.1219 0.1262	97.52 100.96 100.96 97.52 100.96
	0.250	0.5155	14.50 14.25 14.50 14.25 14.75	0.5150	14.50	0.5150 0.5062 0.5150 0.5062 0.5239	0.2495 0.2407 0.2495 0.2407 0.2584	99.80 96.28 99.80 96.28 103.36
	0.375	0.6405	18.50 18.00 18.50 18.25 18.00	0.6400	18.25	0.6488 0.6313 0.6488 0.6400 0.6313	0.3833 0.3658 0.3833 0.3745 0.3658	102.23 97.56 102.23 99.88 97.56
	0.500	0.7655	20.50 20.75 21.25 21.00 21.25	0.7650	21.00	0.7468 0.7559 0.7741 0.7650 0.7741	0.4813 0.4904 0.5086 0.4995 0.5086	96.26 98.08 101.72 99.90 101.72
	0.750	1.0155	27.00 27.75 28.00 27.50 28.00	1.0150	27.70	0.9893 1.0168 1.0259 1.0076 1.0259	0.7238 0.7513 0.7604 0.7421 0.7604	96.48 100.15 101.36 98.92 101.36

Table XIV

Declomycin Capsule

Amount of preparation mg/10 ml	Demethyl Chlorotetracycline HCl (98.12%)							
	Amount added mg	Total amount present mg/10 ml	Diffusion current	Amount of pure mg/10 ml	Average diffusion current	Total amount found mg/10 ml	Amount recovered mg	% Recovery
0.50 mg $=\frac{150x0.5x1.0515}{345.40}$ =0.2283 mg DMCT HCl =0.2327 DMCT HCl (as 98.12%)	0.125	0.3577	11.00 11.00 11.25 11.00 11.00	0.3575	11.00	0.3575 0.3575 0.3656 0.3575 0.3575	0.1248 0.1248 0.1329 0.1248 0.1248	99.84 99.84 106.32 99.84 99.84
	0.250	0.4827	15.00 14.75 15.00 14.75 15.00	0.4825	14.80	0.4890 0.4808 0.4890 0.4808 0.4890	0.2563 0.2481 0.2563 0.2481 0.2563	102.52 99.24 102.52 99.24 102.52
	0.375	0.6077	18.50 18.25 18.50 18.25 18.00	0.6075	18.25	0.6159 0.6075 0.6159 0.6075 0.5992	0.3832 0.3748 0.3832 0.3748 0.3665	102.20 99.96 102.20 99.96 97.75
	0.500	0.7327	22.25 22.75 22.00 22.25 22.50	0.7325	22.20	0.7342 0.7508 0.7260 0.7342 0.7425	0.5015 0.5181 0.4933 0.5015 0.5098	100.30 103.62 98.66 100.30 101.96
	0.750	0.9827	29.25 30.25 29.25 30.00 29.50	0.9825	29.50	0.9740 1.0073 0.9740 0.9990 0.9825	0.7413 0.7746 0.7413 0.7663 0.7498	98.82 103.25 98.82 103.25 99.95

Table XV

Declomycin Syrup

Amount of preparation ml/10 ml	Demethyl Chlorotetracycline HCl (98.12%)							
	Amount added mg	Total amount present mg/10 ml	Diffusion current	Amount of pure mg/10 ml	Average diffusion current	Total amount found mg/10 ml	Amount recovered mg	% Recovery
0.02 ml $\equiv\frac{15\times0.02\times1.0471}{1}$ =0.3141 mg DMCT HCl ≡0.3201 mg DMCT HCl (as 98.12%)	0.125	0.4451	14.25 14.00 14.00 14.25 14.00	0.445	14.10	0.4497 0.4418 0.4418 0.4497 0.4418	0.1296 0.1217 0.1217 0.1296 0.1217	103.68 97.36 97.36 103.68 97.36
	0.250	0.5701	17.50 17.75 17.50 17.25 17.50	0.570	17.50	0.5700 0.5781 0.5700 0.5618 0.5700	0.2499 0.2580 0.2499 0.2417 0.2499	99.96 103.20 99.96 96.68 99.96
	0.375	0.6951	21.75 21.50 21.25 21.25 21.25	0.695	21.40	0.7064 0.6983 0.6902 0.6902 0.6902	0.3863 0.3782 0.3701 0.3701 0.3701	103.03 100.87 98.71 98.71 98.71
	0.500	0.8201	25.00 25.50 25.00 24.75 25.50	0.820	25.00	0.8200 0.8364 0.8200 0.8118 0.8364	0.4999 0.5163 0.4999 0.4917 0.5163	99.98 103.26 99.98 98.34 103.26
	0.750	1.0701	32.50 31.75 32.00 32.75 32.25	1.070	32.20	1.0800 1.0550 1.0634 1.0883 1.0717	0.7599 0.7349 0.7433 0.7682 0.7516	101.29 97.96 99.08 102.40 100.19

Table XVI

Declostatin Capsule

Amount of preparation mg/10 ml	Demethyl Chlorotetracycline HCl (98.12%)							
	Amount added mg	Total amount present mg/10 ml	Diffusion current	Amount of pure mg/10 ml	Average diffusion current	Total amount found mg/10 ml	Amount recovered mg	% Recovery
0.40 mg ≡ 150x0.40x1.052 / 276.12 =0.2286 mg DMCT HCl ≡0.2330 mg DMCT HCl (as 98.12%)	0.125	0.358	11.125 11.25 11.25 11.25 11.25	0.3575	11.25	0.3536 0.3575 0.3575 0.3575 0.3575	0.1206 0.1245 0.1245 0.1245 0.1245	99.48 99.60 99.60 99.60 99.60
	0.250	0.483	15.25 15.00 15.25 15.00 14.75	0.4825	15.00	0.4906 0.4825 0.4906 0.4825 0.4745	0.2576 0.2495 0.2576 0.2495 0.2415	103.04 99.80 103.04 99.80 96.60
	0.375	0.608	18.50 18.25 18.00 18.25 18.00	0.6075	18.15	0.6192 0.6108 0.6025 0.6108 0.6025	0.3862 0.3778 0.3695 0.3778 0.3695	103.00 100.76 98.55 100.76 98.55
	0.500	0.733	22.25 23.00 22.25 22.50 22.50	0.7325	22.50	0.7245 0.7489 0.7245 0.7325 0.7325	0.4915 0.5159 0.4915 0.4995 0.4995	98.30 103.18 98.30 99.90 99.90
	0.750	0.983	30.00 29.25 29.75 29.25 29.50	0.9825	29.45	1.0008 0.9758 0.9925 0.9758 0.9841	0.7678 0.7428 0.7595 0.7428 0.7511	102.35 99.02 101.24 99.02 100.12

Table XVII

Terramycin Capsule

Amount of preparation mg/10 ml	Oxytetracycline HCl (98.17%)							
	Amount added mg	Total amount present mg/10 ml	Diffusion current	Amount of pure mg/10 ml	Average diffusion current	Total amount found mg/10 ml	Amount recovered mg	% Recovery
0.75 mg =250x0.75x1.0094 / 710.67 =0.2663 mg Oxytetra-cycline base =0.2874 mg Oxytetra-cycline HCl =0.2927 mg Oxytetra-cycline HCl (as 98.17%)	0.125	0.4177	12.00 12.00 12.125 12.00 12.00	0.4170	12.00	0.4170 0.4170 0.4213 0.4170 0.4170	0.1243 0.1243 0.1286 0.1243 0.1243	99.44 99.44 102.88 99.44 99.44
	0.250	0.5427	15.50 15.75 15.25 15.50 15.50	0.5420	15.55	0.5402 0.5490 0.5315 0.5402 0.5402	0.2475 0.2563 0.2388 0.2475 0.2475	99.00 102.52 95.52 99.00 99.00
	0.375	0.6677	19.25 19.25 19.00 18.75 19.25	0.6670	19.15	0.6705 0.6705 0.6618 0.6531 0.6705	0.3778 0.3778 0.3691 0.3604 0.3778	100.75 100.75 98.43 96.11 100.75
	0.500	0.7927	22.00 22.50 22.00 22.50 22.25	0.7920	22.25	0.7832 0.8010 0.7832 0.8010 0.7920	0.4905 0.5083 0.4905 0.5083 0.4993	98.10 101.66 98.10 101.66 99.86
	0.750	1.0427	29.75 29.00 30.00 29.75 29.00	1.0420	29.50	1.0508 1.0243 1.0596 1.0508 1.0243	0.7581 0.7316 0.7669 0.7581 0.7316	101.08 97.54 102.25 101.08 97.54

Table XVIII

Terramycin I.V. Powder

Amount of preparation mg/10 ml	Oxytetracycline HCl (98.17%)							
	Amount added mg	Total amount present mg/10 ml	Diffusion current	Amount of pure mg/10 ml	Average diffusion current	Total amount found mg/10 ml	Amount recovered mg	% Recovery
1.50 mg $=\frac{250x1.5x0.9959}{1446.30}$ =0.2582 mg Oxytetra- cycline HCl =0.2630 mg Oxytetra- cycline HCl (as 98.17%)	0.125	0.388	10.75 10.75 10.875 10.75 10.75	0.3875	10.75	0.3875 0.3875 0.3920 0.3875 0.3875	0.1245 0.1245 0.1290 0.1245 0.1245	99.60 99.60 103.20 99.60 99.60
	0.250	0.513	13.75 14.00 14.00 13.75 14.00	0.5125	14.00	0.5034 0.5125 0.5125 0.5034 0.5125	0.2404 0.2495 0.2495 0.2404 0.2495	96.16 99.80 99.80 96.16 99.80
	0.375	0.638	17.00 17.50 17.25 17.00 17.50	0.6375	17.20	0.6300 0.6486 0.6393 0.6300 0.6486	0.3670 0.3856 0.3763 0.3670 0.3856	97.88 102.83 100.35 97.88 102.83
	0.500	0.763	21.75 21.25 21.25 21.50 21.25	0.7625	21.45	0.7732 0.7554 0.7554 0.7643 0.7554	0.5102 0.4924 0.4924 0.5013 0.4924	102.04 98.48 98.48 100.26 98.48
	0.750	1.013	28.00 27.25 28.25 27.00 27.50	1.0125	27.70	1.0234 0.9960 1.0325 0.9868 1.0051	0.7604 0.7330 0.7695 0.7238 0.7421	101.38 97.73 102.60 96.50 98.94

Table XIX

Terramycin I.M. Solution

Amount of preparation ml/10 ml	Oxytetracycline HCl (98.17%)							
	Amount added mg	Total amount present mg/10 ml	Diffusion current	Amount of pure mg/10 ml	Average diffusion current	Total amount found mg/10 ml	Amount recovered mg	% Recovery
0.005 ml $=\frac{100 \times 0.005 \times 0.9996}{2}$ =0.2499 mg Oxytetracycline	0.125	0.3996	10.875 11.00 11.00 10.875 11.00	0.400	11.00	0.3954 0.4000 0.4000 0.3954 0.4000	0.1208 0.1254 0.1254 0.1208 0.1254	96.64 100.32 100.32 96.64 100.32
	0.250	0.5246	15.00 15.00 14.75 15.25 14.75	0.525	15.00	0.5250 0.5250 0.5162 0.5338 0.5162	0.2504 0.2504 0.2416 0.2592 0.2416	100.16 100.16 96.64 103.68 96.64
=0.2696 mg Oxytetracycline HCl	0.375	0.6496	17.75 18.00 17.50 17.75 17.50	0.650	17.70	0.6518 0.6610 0.6426 0.6518 0.6426	0.3772 0.3864 0.3680 0.3775 0.3680	100.59 103.04 98.13 100.67 98.13
=0.2746 mg Oxytetracycline HCl (as 98.17%)	0.500	0.7746	21.75 21.50 22.25 21.75 22.25	0.775	21.95	0.7680 0.7592 0.7856 0.7680 0.7856	0.4934 0.4846 0.5110 0.4934 0.5110	98.68 96.92 102.20 98.68 102.20
	0.750	1.0246	28.25 28.50 28.75 29.00 28.75	1.025	28.75	1.0071 1.0160 1.0250 1.0338 1.0250	0.7325 0.7414 0.7504 0.7592 0.7504	97.66 98.85 100.05 101.22 100.05

Table XX

Terramycin Otic (Solution)

Amount of preparation mg/10 ml	Oxytetracycline HCl (98.17%)							
	Amount added mg	Total amount present mg/10 ml	Diffusion current	Amount of pure mg/10 ml	Average diffusion current	Total amount found mg/10 ml	Amount recovered mg	% Recovery
3.0 mg ≡ 25x3x0.9879 / 272 =0.2724 mg Oxytetracycline ≡0.2939 mg Oxytetracycline HCl ≡0.2994 mg Oxytetracycline HCl (as 98.17%)	0.125	0.4244	12.50 12.625 12.50 12.375 12.50	0.4250	12.50	0.4250 0.4292 0.4250 0.4208 0.4250	0.1256 0.1298 0.1256 0.1214 0.1256	100.48 103.84 100.48 97.12 100.48
	0.250	0.5494	16.00 15.50 15.75 15.75 15.50	0.5500	15.75	0.5587 0.5413 0.5500 0.5500 0.5413	0.2593 0.2419 0.2506 0.2506 0.2419	103.72 96.76 100.24 100.24 96.76
	0.375	0.6744	19.25 18.75 19.00 19.00 18.75	0.6750	19.05	0.6820 0.6643 0.6732 0.6732 0.6643	0.3826 0.3649 0.3738 0.3738 0.3649	102.03 97.32 99.69 99.69 97.32
	0.500	0.7994	23.50 23.75 23.00 24.00 23.50	0.8000	23.50	0.8000 0.8084 0.7829 0.8170 0.8000	0.5006 0.5090 0.4835 0.5176 0.5006	100.12 101.80 96.70 103.52 100.12
	0.750	1.0494	30.25 29.75 29.50 30.50 30.00	1.0500	29.90	1.0624 1.0448 1.0360 1.0712 1.0536	0.7630 0.7454 0.7366 0.7718 0.7542	101.71 99.36 98.19 102.88 100.53

Table XXI

Terramycin Vaginal Tablet

Amount of preparation mg/10 ml	Oxytetracycline HCl (98.17%)							
	Amount added mg	Total amount present mg/10 ml	Diffusion current	Amount of pure mg/10 ml	Average diffusion current	Total amount found mg/10 ml	Amount recovered mg	% Recovery
3.00 mg $=\frac{100x3x0.9492}{1317.65}$ =0.2161 mg Oxytetra- cycline =0.2332 mg Oxytetra- cycline HCl =0.2375 mg Oxytetracycline HCl (as 98.17%)	0.125	0.3625	10.00 10.125 10.00 10.125 10.125	0.3625	10.10	0.3589 0.3634 0.3589 0.3634 0.3634	0.1214 0.1259 0.1214 0.1259 0.1259	97.12 100.72 97.12 100.72 100.72
	0.250	0.4875	14.00 14.00 13.75 14.00 14.25	0.4875	14.00	0.4875 0.4875 0.4788 0.4875 0.4962	0.2500 0.2500 0.2413 0.2500 0.2587	100.00 100.00 96.52 100.00 103.48
	0.375	0.6125	17.25 17.25 17.75 17.50 17.50	0.6125	17.50	0.6038 0.6038 0.6212 0.6125 0.6125	0.3663 0.3663 0.3837 0.3750 0.3750	97.68 97.68 102.32 100.00 100.00
	0.500	0.7375	21.00 21.50 21.25 21.75 21.75	0.7375	21.55	0.7186 0.7357 0.7272 0.7443 0.7443	0.4811 0.4982 0.4897 0.5068 0.5068	96.22 99.64 97.94 101.36 101.36
	0.750	0.9875	28.75 28.25 28.00 28.50 28.00	0.9875	28.40	0.9996 0.9822 0.9736 0.9909 0.9736	0.7621 0.7447 0.7361 0.7534 0.7361	101.61 99.29 98.14 100.45 98.14

Table XXII

Terramycin Pediatric Drops

Amount of preparation ml/10 ml	Calcium Diterramycin (87.13%)							
	Amount added mg	Total amount present mg/10 ml	Diffusion current	Amount of pure mg/10 ml	Average diffusion current	Total amount found mg/10 ml	Amount recovered mg	% Recovery
0.0025 ml $=\frac{100 \times 0.0025 \times 1.0077}{1}$ =0.2519 mg Oxytetra- cycline	0.125	0.4266	11.75 11.75 11.875 11.75 11.875	0.4250	11.75	0.4250 0.4250 0.4295 0.4250 0.4295	0.1234 0.1234 0.1279 0.1234 0.1279	98.72 98.72 102.32 98.72 102.32
	0.250	0.5516	15.50 15.25 15.75 15.50 15.25	0.5500	15.45	0.5518 0.5429 0.5607 0.5518 0.5429	0.2502 0.2413 0.2591 0.2502 0.2413	100.08 96.52 103.64 100.08 96.52
=0.2628 mg Ca-diterra- mycin	0.375	0.6766	18.50 18.00 18.25 18.50 18.25	0.6750	18.30	0.6824 0.6639 0.6732 0.6824 0.6732	0.3808 0.3623 0.3716 0.3808 0.3716	101.55 96.61 99.09 101.55 99.09
=0.3016 mg Ca-diterra- mycin (as 87.13%)	0.500	0.8016	21.50 21.75 22.25 22.00 22.25	0.8000	22.00	0.7818 0.7909 0.8091 0.8000 0.8091	0.4802 0.4893 0.5075 0.4984 0.5075	96.04 97.86 101.50 99.68 101.50
	0.750	1.0516	28.75 28.25 28.00 28.50 28.00	1.0500	28.45	1.0612 1.0427 1.0335 1.0519 1.0335	0.7596 0.7411 0.7319 0.7503 0.7319	101.28 98.81 97.58 100.04 97.58

Table XXIII

Chloromycetin Ophthalmic

Amount of preparation mg/10 ml	Chloramphenicol							
	Amount added mg	Total amount present mg/10 ml	Diffusion current	Amount of pure mg/10 ml	Average diffusion current	Total amount found mg/10 ml	Amount recovered mg	% Recovery
1.00 mg $=\frac{25 \times 1.01474 \times 1}{126.50}$ =0.2006 mg Chloram- phenicol	0.125	0.3256	28.00 28.25 28.50 28.00 28.25	0.325	28.15	0.3231 0.3260 0.3289 0.3231 0.3260	0.1225 0.1254 0.1283 0.1225 0.1254	98.00 100.32 102.64 98.00 100.32
	0.250	0.4506	36.75 36.00 36.50 36.25 36.50	0.450	36.30	0.4557 0.4464 0.4526 0.4495 0.4526	0.2551 0.2458 0.2520 0.2489 0.2520	102.04 98.32 100.80 99.56 100.80
	0.375	0.5756	46.50 46.75 45.25 45.75 45.50	0.575	46.00	0.5812 0.5844 0.5656 0.5719 0.5688	0.3806 0.3838 0.3650 0.3713 0.3682	101.51 102.36 97.35 99.03 98.20
	0.500	0.7006	57.50 55.75 56.25 57.50 56.75	0.700	56.80	0.7084 0.6868 0.6930 0.7084 0.6992	0.5078 0.4862 0.4924 0.5078 0.4986	101.56 97.24 98.48 101.56 99.72
	0.625	0.8256	65.75 67.75 66.50 66.25 65.50	0.825	66.25	0.8186 0.8435 0.8279 0.8250 0.8155	0.6180 0.6429 0.6273 0.6244 0.6149	98.88 102.86 100.37 99.90 98.38

Table XXIV

Erythrocin Chewable Tablet

Amount of preparation mg/10 ml	Erythromycin Ethyl Succinate							
	Amount added mg	Total amount present mg/10 ml	Diffusion current	Amount of pure mg/10 ml	Average diffusion current	Total amount found mg/10 ml	Amount recovered mg	% Recovery
0.5 mg ≒200x0.5x1.0499 / 741.60 ≒0.1416 mg Erythro-mycin ≒0.1750 mg Erythro-mycin Ethyl Succinate	0.10	0.275	35.00	0.275	35.05	0.2746	0.0996	99.60
			34.75			0.2726	0.0989	98.90
			35.50			0.2785	0.1035	103.50
			35.25			0.2766	0.1016	101.60
			35.25			0.2766	0.1016	101.60
	0.20	0.375	47.00	0.375	47.20	0.3734	0.1984	99.20
			46.50			0.3694	0.1944	97.20
			48.00			0.3814	0.2064	103.20
			47.50			0.3774	0.2024	101.20
			46.75			0.3714	0.1964	98.20
	0.30	0.475	61.00	0.475	59.80	0.4845	0.3066	102.21
			58.75			0.4666	0.2916	97.20
			59.00			0.4686	0.2936	97.87
			60.50			0.4806	0.3056	101.87
			59.75			0.4746	0.2996	99.87
	0.35	0.525	66.00	0.525	66.00	0.5250	0.3500	100.00
			67.50			0.5369	0.3619	103.39
			66.75			0.5310	0.3560	101.71
			64.75			0.5150	0.3400	97.14
			65.25			0.5190	0.3440	98.28
	0.40	0.575	70.25	0.575	72.00	0.5610	0.3860	96.50
			71.00			0.5670	0.3920	98.00
			73.50			0.5870	0.4120	103.00
			72.75			0.5810	0.4060	101.50
			72.00			0.5750	0.4000	100.00

Average = 100.11±2.19

Table XXV

Erythrocin Granules

Amount of preparation mg/10 ml	Erythromycin Ethyl Succinate							
	Amount added mg	Total amount present mg/10 ml	Diffusion current	Amount of pure mg/10 ml	Average diffusion current	Total amount found mg/10 ml	Amount recovered mg	% Recovery
1.0 mg $=\frac{2.4 \times 1 \times 1.044}{21.1993}$ =0.1182 mg Erythro-mycin Ethyl Succinate	0.10	0.2182	28.00	0.22	28.60	0.2154	0.0972	97.20
			28.50			0.2192	0.1010	101.00
			28.25			0.2173	0.0991	99.10
			28.75			0.2212	0.1030	103.00
			28.00			0.2154	0.0972	97.20
	0.15	0.2682	34.25	0.27	35.00	0.2642	0.1460	97.33
			34.50			0.2661	0.1479	98.60
			35.25			0.2719	0.1537	102.47
			34.75			0.2681	0.1499	99.93
			34.50			0.2661	0.1479	98.60
	0.20	0.3182	40.75	0.32	40.95	0.3184	0.2002	100.10
			41.00			0.3204	0.2022	101.10
			40.00			0.3126	0.1944	97.20
			40.75			0.3184	0.2002	100.10
			41.50			0.3243	0.2061	103.05
	0.25	0.3682	46.50	0.37	47.00	0.3661	0.2479	99.16
			47.25			0.3720	0.2538	101.52
			46.00			0.3621	0.2439	97.56
			47.50			0.3739	0.2557	102.28
			46.25			0.3641	0.2459	98.36
	0.30	0.4182	51.25	0.42	52.10	0.4131	0.2949	98.30
			53.00			0.4272	0.3090	103.00
			52.50			0.4232	0.3050	101.67
			51.50			0.4152	0.2970	99.00
			51.00			0.4111	0.2929	97.63

Average = 99.78±2.03

Table XXVI

Erythrocin I.M.

Amount of preparation ml/10 ml	Erythromycin Ethyl Succinate							
	Amount added mg	Total amount present mg/10 ml	Diffusion current	Amount of pure mg/10 ml	Average diffusion current	Total amount found mg/10 ml	Amount recovered mg	% Recovery
0.005 ml $=\frac{50 \times 0.005 \times 1.0039}{1}$ =0.2510 mg Erythromycin =0.3101 mg Erythromycin Ethyl Succinate	0.05	0.3601	45.75 46.00 45.75 46.00 45.75	0.36	45.80	0.3596 0.3616 0.3596 0.3616 0.3596	0.0495 0.0515 0.0495 0.0515 0.0495	99.00 103.00 99.00 103.00 99.00
	0.10	0.4101	52.50 51.75 52.00 52.25 51.75	0.41	52.00	0.4139 0.4080 0.4100 0.4120 0.4080	0.1038 0.0979 0.0999 0.1019 0.0979	103.80 97.90 99.90 101.90 97.90
	0.15	0.4601	57.50 58.00 58.25 57.25 57.75	0.46	57.75	0.4580 0.4620 0.4640 0.4560 0.4600	0.1479 0.1519 0.1539 0.1459 0.1499	98.60 101.27 102.60 97.27 99.93
	0.20	0.5101	63.75 65.00 64.25 65.25 64.50	0.51	64.50	0.5041 0.5140 0.5080 0.5159 0.5100	0.1940 0.2039 0.1979 0.2058 0.1999	97.00 101.95 98.95 102.90 99.95
	0.25	0.5601	70.25 71.00 69.50 70.00 70.75	0.56	70.15	0.5608 0.5668 0.5548 0.5588 0.5648	0.2507 0.2567 0.2447 0.2487 0.2547	100.28 102.68 97.88 99.48 101.88

Average = 100.28±2.06

Table XXVII

Olicin

Amount of preparation mg/10 ml	Triacetyl Oleandomycin (98.70%)							
	Amount added mg	Total amount present mg/10 ml	Diffusion current	Amount of pure mg/10 ml	Average diffusion current	Total amount found mg/10 ml	Amount recovered mg	% Recovery
0.25 mg =250x0.25x1.0016 648.65 =0.0965 mg Oleandomycin =0.1138 mg Triacetyl Oleandomycin =0.1153 mg Triacetyl Oleandomycin (as 98.70%)	0.05	0.1653	35.75 35.25 35.50 35.00 35.25	0.165	35.25	0.1673 0.1650 0.1662 0.1638 0.1650	0.0520 0.0497 0.0509 0.0485 0.0497	104.00 99.40 101.80 97.00 99.40
	0.10	0.2153	45.75 46.00 45.25 45.00 45.25	0.215	45.40	0.2166 0.2178 0.2143 0.2131 0.2143	0.1013 0.1025 0.0990 0.0978 0.0990	101.30 102.50 99.00 97.80 99.00
	0.15	0.2653	56.50 56.25 57.75 57.00 56.50	0.265	57.00	0.2627 0.2615 0.2685 0.2662 0.2627	0.1474 0.1462 0.1532 0.1509 0.1474	98.27 97.48 102.13 100.60 98.27
	0.20	0.3153	67.92 67.08 67.50 66.25 67.08	0.315	67.08	0.3190 0.3150 0.3170 0.3111 0.3150	0.2037 0.1997 0.2017 0.1958 0.1997	101.85 99.85 100.85 97.90 99.85
	0.25	0.3653	76.25 78.75 78.33 77.08 77.08	0.365	77.58	0.3588 0.3705 0.3685 0.3627 0.3627	0.2435 0.2552 0.2532 0.2474 0.2474	97.40 102.08 101.28 98.96 98.96

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